

SALTS AND SOLID FORMS OF COMPOUNDS THAT MODULATE IKZF2**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of United States Provisional Application Serial Number 63/516,062, filed July 27, 2023, the contents of which is hereby incorporated by reference in its entirety.

FIELD

[0002] The present disclosure relates generally to salts and solid forms of compounds that bind to cereblon, thereby modulating cereblon activity. In some embodiments, compounds disclosed herein bind to cereblon, thereby initiating degradation of IKZF proteins (e.g., IKZF2).

BACKGROUND

[0003] IKAROS Family Zinc Finger 2 (IKZF2) (also known as Helios) is one of the five members of the Ikaros family of transcription factors found in mammals. IKZF2 is a critical regulator of T cell activity and function. Genetic deletion of Helios resulted in an enhanced anti-tumor immune response (Kim et al., Science 350:334-339 (2015)). Notably, Helios is highly expressed in regulatory T cells (Tregs) (Elkord et al., Expert Opin. Biol. Ther. 12:1423-1425 (2012)), a subpopulation of T cells that restricts the activity of effector T cells. Selective deletion of Helios in regulatory T cells resulted in both loss of suppressive activity and acquisition of effector T cell functions (Najagawa et al., Proc. Natl. Acad. Sci. USA 113:6248-6253 (2016); Yates et al., Proc. Natl. Acad. Sci. USA 115:2162-2167 (2018)). Therefore, Helios is a critical factor in restricting T cell effector function in Tregs.

[0004] Helios expression has also been reported to be upregulated in “exhausted” T cells, in the settings of both chronic viral infections (Crawford et al., Immunity 40:289-302 (2014), Doering et al., Immunity 37:1130-1144 (2012); Scott-Browne et al., Immunity 45:1327-1340 (2016)) and tumors (Martinez et al., Immunity 42:265-278 (2015); Mognol et al., Proc. Natl. Acad. Sci. USA 114:E2776-E2785 (2017); Pereira et al., J. Leukoc. Biol. 102:601-615 (2017); Singer et al., Cell 166:1500-1511 (2016); Schietinger et al., Immunity 45:389-401 (2016)), as well as in dysfunctional chimeric antigen receptor (CAR) T cells (Long et al., Nat. Med. 21:581-590 (2015)). Overexpression or aberrant expression of Helios and various splice isoforms have been reported in several hematological malignancies, including T cell leukemias and lymphomas (Nakase et al., Exp. Hematol. 30:313-317 (2002); Tabayashi et al., Cancer Sci. 98:182-188 (2007); Asanuma et al., Cancer Sci. 104:1097-1106 (2013)). Moreover, knockdown of Helios in a model of mixed lineage leukemia (MLL)-driven myeloid leukemia potently suppressed proliferation and increased cell death (Park et al., J. Clin. Invest. 125:1286-1298 (2015); Park et al., Cell Stem Cell 24:153-165 (2019)).

[0005] Currently, anti-CTLA4 antibodies are used in the clinic to target Tregs in tumors. However, targeting CTLA4 often causes systemic activation of T-effector cells, resulting in excessive toxicity and limiting therapeutic utility. Up to 75% of patients treated with a combination of anti-PD1 and anti-CTLA4 have reported grade 3 or higher adverse events (National Cancer Institute, Division of Cancer Treatment &

diagnosis, Common Terminology for Adverse Events (CTCAE), https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

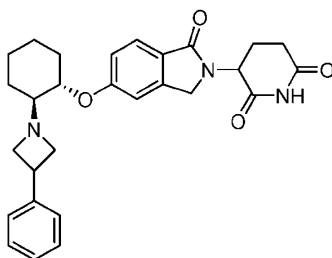
[0006] There is a need for therapies that can target Tregs in tumors without causing systemic activation of T-effector cells. Accordingly, an IKZF2-specific modulator or degrader would have the potential to focus an enhanced immune response to areas within or near tumors providing a potentially more tolerable and less toxic therapy for the treatment of diseases mediated by IKZF2.

SUMMARY

[0007] Provided herein are salts and solid forms of compounds that bind to cereblon and are useful as potential therapeutic agents for the treatment of diseases mediated by IKZF2. Also provided herein are crystalline forms of Compound I and Compound II which exhibit superior pharmacokinetic properties and chemical and physical stability under various conditions.

[0008] Also disclosed herein are pharmaceutical compositions comprising salts and solid forms of Compound I and Compound II and methods of using the same in the treatment of IKZF2-mediated diseases or disorders.

[0009] In one embodiment, provided herein is a crystalline form of Compound I:



I,

(Compound I Form A), wherein Compound I Form A is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 13.7, 17.5, and 21.4 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0010] In one embodiment, provided herein is an amorphous form of Compound I (Compound I Amorphous Form), wherein Compound I amorphous Form is characterized by an X-ray powder diffractogram substantially as shown in FIG 4.

[0011] In one embodiment, provided herein is a fumarate salt of Compound I.

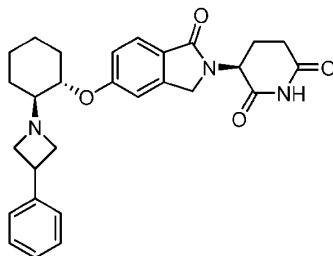
[0012] In one embodiment, provided herein is a L-tartrate salt of Compound I.

[0013] In one embodiment, provided herein is a nicotinamide co-crystal of Compound I.

[0014] In one embodiment, provided herein is a succinate salt of Compound I.

[0015] In one embodiment, provided herein is a R-mandelate salt of Compound I.

[0016] In one embodiment, provided herein is an amorphous form of Compound II:



II

(Compound II Amorphous Form), wherein Compound II amorphous Form is characterized by an X-ray powder diffractogram substantially as shown in FIG 18.

[0017] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form B), wherein Compound II Form B is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.3, 17.8, and 19.0 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0018] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form C), wherein Compound II Form C is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 4.0, 12.0, and 14.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0019] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form D1), wherein Compound II Form D1 is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4, 7.9, and 15.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0020] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form D2), wherein Compound II Form D2 is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4, 16.2, and 17.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0021] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form D3), wherein Compound II Form D3 is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4, 8.1, and 16.2 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0022] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form E), wherein Compound II Form E is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.1, 7.6, and 15.2 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0023] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form F), wherein Compound II Form F is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.2, 18.0, and 19.2 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0024] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form G), wherein Compound II Form G is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.3, 18.4, and 19.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0025] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form H), wherein Compound II Form H is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.2, 18.1, and 21.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0026] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form I), wherein Compound II Form I is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 15.4, 15.8, and 21.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0027] In one embodiment, provided herein is a crystalline form of Compound II (Compound II Form J), wherein Compound II Form J is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 17.2, 17.6, and 20.4 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0028] In one embodiment, provided herein is a fumarate salt of Compound II.

[0029] Also provided herein is a pharmaceutical composition comprising an effective amount of a salt or solid form of a compound disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer, or tautomer thereof, and a pharmaceutically acceptable excipient.

[0030] Also provided herein are methods for modulating cereblon activity which method comprises contacting cereblon with an effective amount of a salt or solid form of a compound disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer, or tautomer thereof.

[0031] Also provided herein are methods for degrading IKZF2, which method comprises contacting IKZF2 with an effective amount of a salt or solid form of a compound disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer, or tautomer thereof.

[0032] Also provided herein are methods to treat cancer in a subject in need thereof, which method comprises selecting a subject whose cancer is mediated at least in part by IKZF2 and administering to said subject an effective amount of a salt or solid form of a compound disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer, or tautomer thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1 shows an X-ray powder diffraction (XRPD) pattern of Compound I Form A.

[0034] FIG. 2 shows a differential scanning calorimetry (DSC) curve of Compound I Form A.

[0035] FIG. 3 shows thermogravimetric analysis (TGA) of Compound I Form A.

[0036] FIG. 4 shows an XRPD pattern of Compound I Amorphous Form.

[0037] FIG. 5 shows an XRPD pattern of Compound I fumarate salt Form A.

[0038] FIG. 6 shows a DSC curve of Compound I fumarate salt Form A.

[0039] FIG. 7 shows a TGA of Compound I fumarate salt Form A.

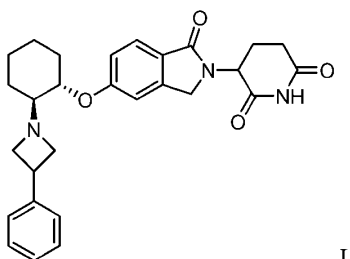
[0040] FIG. 8 shows an XRPD pattern of Compound I L-tartrate salt Form A.

- [0041] FIG. 9 shows a DSC curve of Compound I L-tartrate salt Form A.
- [0042] FIG. 10 shows a TGA of Compound I L-tartrate salt Form A.
- [0043] FIG. 11 shows an XRPD pattern of Compound I nicotinamide co-crystal Form A.
- [0044] FIG. 12 shows an XRPD pattern of Compound I succinate salt Form A.
- [0045] FIG. 13 shows a DSC curve of Compound I succinate salt Form A.
- [0046] FIG. 14 shows a TGA of Compound I succinate salt Form A.
- [0047] FIG. 15 shows an XRPD pattern of Compound I R-mandelate salt Form A.
- [0048] FIG. 16 shows a DSC curve of Compound I R-mandelate salt Form A.
- [0049] FIG. 17 shows a TGA of Compound I R-mandelate salt Form A.
- [0050] FIG. 18 shows an XRPD pattern of Compound II Amorphous Form.
- [0051] FIG. 19 shows an XRPD pattern of Compound II Form B.
- [0052] FIG. 20 shows a DSC curve of Compound II Form B.
- [0053] FIG. 21 shows a TGA of Compound II Form B.
- [0054] FIG. 22 shows an XRPD pattern of Compound II Form C.
- [0055] FIG. 23 shows a DSC curve of Compound II Form C.
- [0056] FIG. 24 shows a TGA of Compound II Form C.
- [0057] FIG. 25 shows an XRPD pattern of Compound II Form D1.
- [0058] FIG. 26 shows a DSC curve of Compound II Form D1.
- [0059] FIG. 27 shows a TGA of Compound II Form D1.
- [0060] FIG. 28 shows an XRPD pattern of Compound II Form D2.
- [0061] FIG. 29 shows a DSC curve of Compound II Form D2.
- [0062] FIG. 30 shows an XRPD pattern of Compound II Form D3.
- [0063] FIG. 31 shows a DSC curve of Compound II Form D3.
- [0064] FIG. 32 shows a TGA of Compound II Form D3.
- [0065] FIG. 33 an XRPD pattern of Compound II Form E.
- [0066] FIG. 34 shows a DSC curve of Compound II Form E.
- [0067] FIG. 35 shows a TGA of Compound II Form E.
- [0068] FIG. 36 shows an XRPD pattern of Compound II Form F.
- [0069] FIG. 37 shows a DSC curve of Compound II Form F.

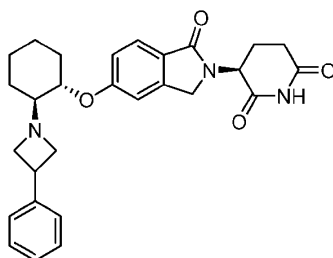
- [0070] FIG. 38 shows a TGA of Compound II Form F.
- [0071] FIG. 39 shows an XRPD pattern of Compound II Form G.
- [0072] FIG. 40 shows a DSC curve of Compound II Form G.
- [0073] FIG. 41 shows a TGA of Compound II Form G.
- [0074] FIG. 42 shows an XRPD pattern of Compound II Form H.
- [0075] FIG. 43 shows a DSC curve of Compound II Form H.
- [0076] FIG. 44 shows a TGA of Compound II Form H.
- [0077] FIG. 45 shows an Ortep image of Compound II Form H single crystal structure from SCXRD-01.
- [0078] FIG. 46 shows an Ortep image of Compound II Form H single crystal structure from SCXRD-02.
- [0079] FIG. 47 shows an Ortep image of Compound II Form H single crystal structure from SCXRD-03.
- [0080] FIG. 48 shows an XRPD pattern of Compound II Form I.
- [0081] FIG. 49 shows a DSC curve of Compound II Form I.
- [0082] FIG. 50 shows a TGA of Compound II Form I.
- [0083] FIG. 51 shows an XRPD pattern of Compound II Form J.
- [0084] FIG. 52 shows a DSC curve of Compound II Form J.
- [0085] FIG. 53 shows an XRPD pattern of Compound II fumarate salt Form B.
- [0086] FIG. 54 shows a DSC curve of Compound II fumarate salt Form B.
- [0087] FIG. 55 shows a TGA of Compound II fumarate salt Form B.
- [0088] FIG. 56 shows an XRPD pattern of Compound II fumarate salt Form C.
- [0089] FIG. 57 shows a TGA of Compound II fumarate salt Form C.

DETAILED DESCRIPTION

[0090] The compound 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione designated herein as Compound I, has the following formula:



[0091] The compound (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione designated herein as Compound II, has the following formula:



II.

[0092] Compound I and Compound II bind to and modulate cereblon. The synthesis and method of use thereof is described in PCT International Application Publication No. WO2023/283425, which is incorporated by reference in its entirety. Unless otherwise specified, reference to Compound I (CAS Registry number 2892065-49-3) and Compound II (2892065-45-9) is intended to encompass the compound per se, or a salt, such as a pharmaceutically acceptable salt, stereoisomer, mixture of stereoisomers, prodrug, solid form, co-crystal, solvate, and/or hydrate thereof.

Definitions

[0093] As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

[0094] The term “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense, that is, as “including, but not limited to.” Further, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus, reference to “the compound” includes a plurality of such compounds, and reference to “the assay” includes reference to one or more assays and equivalents thereof known to those skilled in the art.

[0095] Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. In certain embodiments, the term “about” includes the indicated amount $\pm 10\%$. In other embodiments, the term “about” includes the indicated amount $\pm 5\%$. In certain other embodiments, the term “about” includes the indicated amount $\pm 2.5\%$. In certain other embodiments, the term “about” includes the indicated amount $\pm 1\%$. Also, to the term “about X” includes description of “X”.

[0096] Recitation of numeric ranges of values throughout the disclosure is intended to serve as a shorthand notation of referring individually to each separate value falling within the range inclusive of the values defining the range, and each separate value is incorporated in the specification as it were individually recited herein.

[0097] Forms of Compound I or salts, co-crystals, solvates, or hydrates thereof are provided herein. In one embodiment, reference to a form of Compound I or a salt, co-crystal, solvate, or hydrate thereof means that at least 50% to 99% (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%) of Compound I or a salt, co-crystal, solvate, or hydrate thereof is present in a composition in the designated form. For instance, in one embodiment, reference to Compound I free acid Form A means that at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% of Compound I, as the free acid, is present in a composition as Form A.

[0098] The term “solid form” refers to a type of solid-state material that includes amorphous as well as crystalline forms. The term “crystalline form” refers to polymorphs as well as solvates, hydrates, etc. The term “polymorph” refers to a particular crystal structure having particular physical properties such as X-ray diffraction, melting point, and the like.

[0099] The term “co-crystal” refers to a molecular complex of a compound disclosed herein and one or more non-ionized co-crystal formers connected via non-covalent interactions. In some embodiments, the co-crystals disclosed herein may include a non-ionized form of Compound I (*e.g.*, Compound I free form) and one or more non-ionized co-crystal formers, where non-ionized Compound I and the co-crystal former(s) are connected through non-covalent interactions. In some embodiments, co-crystals disclosed herein may include an ionized form of Compound I (*e.g.*, a salt of Compound I) and one or more non-ionized co-crystals formers, where ionized Compound I and the co-crystal former(s) are connected through non-covalent interactions. Co-crystals may additionally be present in anhydrous, solvated or hydrated forms. In certain instances, co-crystals may have improved properties as compared to the parent form (*i.e.*, the free molecule, zwitterion, etc.) or a salt of the parent compound. Improved properties can be increased solubility, increased dissolution, increased bioavailability, increased dose response, decreased hygroscopicity, increased stability, a crystalline form of a normally amorphous compound, a crystalline form of a difficult to salt or unsalttable compound, decreased form diversity, more desired morphology, and the like. Methods for making and characterizing co-crystals are known to those of skill in the art.

[0100] The term “co-crystal former” or “co-former” refers to one or more pharmaceutically acceptable bases or pharmaceutically acceptable acids disclosed herein in association with Compound I, or any other compound disclosed herein. Such bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such acids include inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, maleic acid, tartaric acid, citric acid, methanesulfonic acid, ethanesulfonic acid, gluconic acid, glutamic acid, salicylic acid, stearic acid, and the like.

[0101] The term “solvate” refers to a complex formed by combination of solvent molecules with molecules or ions of the solute. The solvent can be an organic compound, an inorganic compound, or a mixture of

both. As used herein, the term “solvate” includes a “hydrate” (*i.e.*, a complex formed by combination of water molecules with molecules or ions of the solute), hemi-hydrate, channel hydrate, etc. Some examples of solvents include, but are not limited to, acetonitrile, methanol, N,N-dimethylformamide, tetrahydrofuran, 2-methyltetrahydrofuran, dimethylsulfoxide, and water. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present disclosure.

[0102] The term “desolvated” refers to a Compound I form that is a solvate as described herein, and from which solvent molecules have been partially or completely removed. Desolvation techniques to produce desolvated forms include, without limitation, exposure of a Compound I form (solvate) to a vacuum, subjecting the solvate to elevated temperature, exposing the solvate to a stream of gas, such as air or nitrogen, or any combination thereof. Thus, a desolvated Compound I form can be anhydrous, *i.e.*, completely without solvent molecules, or partially solvated wherein solvent molecules are present in stoichiometric or non-stoichiometric amounts.

[0103] The term “amorphous” refers to a state in which the material lacks long range order at the molecular level and, depending upon temperature, may exhibit the physical properties of a solid or a liquid. Typically such materials do not give distinctive X-ray diffraction patterns and, while exhibiting the properties of a solid, are more formally described as a liquid. Upon heating, a change from solid to liquid properties occurs which is characterized by a change of state, typically second order (glass transition).

[0104] Any formula or structure given herein, including Compound I, is also intended to represent unlabeled forms as well as isotopically labeled forms of the compounds. It is understood that for any given atom, the isotopes may be present essentially in ratios according to their natural occurrence, or one or more particular atoms may be enhanced with respect to one or more isotopes using synthetic methods known to one skilled in the art. Thus, hydrogen includes for example ^1H , ^2H , ^3H ; carbon includes for example ^{11}C , ^{12}C , ^{13}C , ^{14}C ; oxygen includes for example ^{16}O , ^{17}O , ^{18}O ; nitrogen includes for example ^{13}N , ^{14}N , ^{15}N ; sulfur includes for example ^{32}S , ^{33}S , ^{34}S , ^{35}S , ^{36}S , ^{37}S , ^{38}S ; fluoro includes for example ^{17}F , ^{18}F , ^{19}F ; chloro includes for example ^{35}Cl , ^{36}Cl , ^{37}Cl , ^{38}Cl , ^{39}Cl ; and the like.

[0105] A “stereoisomer” refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. The present disclosure contemplates various stereoisomers, or mixtures thereof, and includes “enantiomers,” which refers to two stereoisomers whose molecules are nonsuperimposable mirror images of one another.

[0106] “Diastereomers” are stereoisomers that have at least two asymmetric atoms, but which are not mirror-images of each other.

[0107] The term “tautomer” refers to structurally distinct isomers that interconvert by tautomerization. Tautomerization is a form of isomerization and includes prototropic or proton-shift tautomerization, which is considered a subset of acid-base chemistry. Prototropic tautomerization or proton-shift tautomerization involves the migration of a proton accompanied by changes in bond order, often the interchange of a single

bond with an adjacent double bond. Where tautomerization is possible (e.g. in solution), a chemical equilibrium of tautomers can be reached.

[0108] As used herein, the terms “treat,” “treating,” “therapy,” “therapies,” and like terms refer to the administration of material, *e.g.*, any one or more solid, crystalline or polymorphs of Compound I as described herein in an amount effective to prevent, alleviate, or ameliorate one or more symptoms of a disease or condition, *i.e.*, indication, and/or to prolong the survival of the subject being treated.

[0109] The term “administering” refers to oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, intranasal or subcutaneous administration, or the implantation of a slow-release device *e.g.*, a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.*, buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.*, intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc.

[0110] As used herein, the term “modulating” or “modulate” refers to an effect of altering a biological activity, especially a biological activity associated with a particular biomolecule such as IKZF2 activity. For example, an agonist or antagonist of a particular biomolecule modulates the activity of the IKZF2 by either increasing (*e.g.* agonist, activator), or decreasing (*e.g.* antagonist, inhibitor) the activity, of the biomolecule. Such activity is typically indicated in terms of an inhibitory concentration (IC₅₀) or excitation concentration (EC₅₀) of the compound for an inhibitor or activator, respectively.

[0111] As used herein, the term “composition” refers to a pharmaceutical preparation suitable for administration to an intended subject for therapeutic purposes that contains at least one pharmaceutically active compound, including any solid form thereof. The composition may include at least one pharmaceutically acceptable component to provide an improved formulation of the compound, such as a suitable carrier or excipient.

[0112] As used herein, the term “subject” or “patient” refers to a living organism that is treated with compounds as described herein, including, but not limited to, any mammal, such as a human, other primates, sports animals, animals of commercial interest such as cattle, farm animals such as horses, or pets such as dogs and cats.

[0113] The term “pharmaceutically acceptable” indicates that the indicated material does not have properties that would cause a reasonably prudent medical practitioner to avoid administration of the material to a patient, taking into consideration the disease or conditions to be treated and the respective route of administration. For example, it is commonly required that such a material be essentially sterile, *e.g.*, for injectables. A “pharmaceutically acceptable salt” of a compound indicates a salt that is pharmaceutically acceptable and possesses the desired pharmacological activity of the parent compound, *i.e.*, salts which

retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable.

[0114] In the present context, the term “therapeutically effective” or “effective amount” indicates that the materials or amount of material is effective to prevent, alleviate, or ameliorate one or more symptoms of a disease or medical condition, and/or to prolong the survival of the subject being treated. The therapeutically effective amount will vary depending on the compound, the disorder or condition and its severity and the age, weight, etc., of the mammal to be treated. For example, an effective amount is an amount sufficient to effectuate a beneficial or desired clinical result. The effective amounts can be provided all at once in a single administration or in fractional amounts that provide the effective amount in several administrations. The precise determination of what would be considered an effective amount may be based on factors individual to each subject, including their size, age, injury, and/or disease or injury being treated, and amount of time since the injury occurred or the disease began. One skilled in the art will be able to determine the effective amount for a given subject based on these considerations which are routine in the art.

[0115] In some embodiments, the phrase “substantially shown in Figure” as applied to an X-ray powder diffractogram is meant to include a variation of $\pm 0.2^\circ 2\theta$ or $\pm 0.1^\circ 2\theta$, as applied to DSC thermograms is meant to include a variation of $\pm 3^\circ \text{Celsius}$, and as applied to thermogravimetric analysis (TGA) is meant to include a variation of $\pm 2\%$ in weight loss.

[0116] “Substantially pure form (of a polymorph),” in some embodiments, means that in the referenced material, at least 99.9% of the material is the referenced polymorph. “Substantially pure form (of a polymorph),” in some embodiments, means that in the referenced material, at least 99.5% of the material is the referenced polymorph. “Substantially pure form (of a polymorph),” in some embodiments, means that in the referenced material, at least 99% of the material is the referenced polymorph. “Substantially pure form (of a polymorph),” in some embodiments, means that in the referenced material, at least 98% of the material is the referenced polymorph. “Substantially pure form (of a polymorph),” in some embodiments, means that in the referenced material, at least 97% of the material is the referenced polymorph. “Substantially pure form (of a polymorph),” in some embodiments, means that in the referenced material, at least 96% of the material is the referenced polymorph. “Substantially pure form (of a polymorph),” in some embodiments, means that in the referenced material, at least 95% of the material is the referenced polymorph.

[0117] In the context of the use, testing, or screening of compounds that are or may be modulators, the term “contacting” means that the compound(s) are caused to be in sufficient proximity to a particular molecule, complex, cell, tissue, organism, or other specified material that potential binding interactions and/or chemical reaction between the compound and other specified material can occur.

Salts and Forms of Compound I

[0118] While not intending to be bound by any particular theory, certain solid forms are characterized by physical properties, e.g., stability, solubility, and dissolution rate, appropriate for pharmaceutical and

therapeutic dosage forms. Moreover, while not wishing to be bound by any particular theory, certain solid forms are characterized by physical properties (e.g., density, compressibility, hardness, morphology, cleavage, stickiness, solubility, water uptake, electrical properties, thermal behavior, solid-state reactivity, physical stability, and chemical stability) affecting particular processes (e.g., yield, filtration, washing, drying, milling, mixing, tableting, flowability, dissolution, formulation, and lyophilization) which make certain solid forms suitable for the manufacture of a solid dosage form. Such properties can be determined using particular analytical chemical techniques, including solid-state analytical techniques (e.g., X-ray diffraction, microscopy, spectroscopy, and thermal analysis), as described herein.

[0119] The identification and selection of a solid form of a pharmaceutical compound are complex, given that a change in solid form may affect a variety of physical and chemical properties, which may provide benefits or drawbacks in processing, formulation, stability, bioavailability, storage, and handling (e.g., shipping), among other important pharmaceutical characteristics. Useful pharmaceutical solids include crystalline solids and amorphous solids, depending on the product and its mode of administration. Amorphous solids are characterized by a lack of long-range structural order, whereas crystalline solids are characterized by structural periodicity. The desired class of pharmaceutical solid depends upon the specific application; amorphous solids are sometimes selected on the basis of, e.g., an enhanced dissolution profile, while crystalline solids may be desirable for properties such as, e.g., physical, or chemical stability.

[0120] Whether crystalline or amorphous, solid forms of a pharmaceutical compound include single-component and multiple-component solids. Single-component solids consist essentially of the pharmaceutical compound or active ingredient in the absence of other compounds. Variety among single-component crystalline materials may potentially arise from the phenomenon of polymorphism, wherein multiple three-dimensional arrangements exist for a particular pharmaceutical compound.

[0121] Notably, it is not possible to predict a priori if crystalline forms of a compound even exist, let alone how to successfully prepare them (see, e.g., Braga and Grepioni, 2005, "Making crystals from crystals: a green route to crystal engineering and polymorphism," *Chem. Commun.*:3635-3645 (with respect to crystal engineering, if instructions are not very precise and/or if other external factors affect the process, the result can be unpredictable); Jones et al., 2006, *Pharmaceutical Cocrystals: An Emerging Approach to Physical Property Enhancement*, *MRS Bulletin* 31:875-879 (At present it is not generally possible to computationally predict the number of observable polymorphs of even the simplest molecules); Price, 2004, "The computational prediction of pharmaceutical crystal structures and polymorphism," *Advanced Drug Delivery Reviews* 56:301-319 ("Price"); and Bernstein, 2004, "Crystal Structure Prediction and Polymorphism," *ACA Transactions* 39:14-23 (a great deal still needs to be learned and done before one can state with any degree of confidence the ability to predict a crystal structure, much less polymorphic forms)).

[0122] The variety of possible solid forms creates potential diversity in physical and chemical properties for a given pharmaceutical compound. The discovery and selection of solid forms are of great importance in the development of an effective, stable, and marketable pharmaceutical product.

[0123] As described generally above, the present disclosure provides salts and solid forms (e.g. crystalline or amorphous forms) of the compound, 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (hereinafter “Compound I”). Salts and solid forms (e.g. crystalline or amorphous forms) of Compound I are collectively referred to herein as “forms of Compound I.”

[0124] In some embodiments, Compound I is a free base. In some embodiments, Compound I is a salt. In some embodiments, Compound I is a pharmaceutically acceptable salt. In some embodiments, Compound I is a solvate. In some embodiments, Compound I is a hydrate. In some embodiments, Compound I is unsolvated.

Forms of Compound I

Compound I Form A

[0125] In one embodiment, provided is crystalline 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I Form A), wherein Compound I Form A is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 13.7, 17.5, and 21.4 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0126] In some embodiments, Compound I Form A is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 10.9, 15.5, or 16.4 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 1;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 207.1 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 2;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 0.6 wt% up to 150 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in

FIG. 3.

[0127] In one embodiment, provided is amorphous 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I Amorphous Form), characterized by an X-ray powder diffractogram substantially as shown in FIG. 4.

[0128] In one embodiment, provided is a fumarate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione.

[0129] In some embodiments, provided is crystalline fumarate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I fumarate salt Form A), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 10.0, 18.1, and 23.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0130] In some embodiments, Compound I fumarate salt Form A is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 5.6, 19.7, or 21.8 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 5;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 16.1 $^\circ\text{C}$ (onset temperature) and an endotherm at about 120.0 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 6;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.5 wt% up to 120 $^\circ\text{C}$, and a further weight loss of about 8.3 wt% from 120 $^\circ\text{C}$ up to 173 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 7.

[0131] In one embodiment, provided is a L-tartrate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione.

[0132] In some embodiments, provided is crystalline L-tartrate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I L-tartrate salt Form A), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.9, 17.0, and 22.3 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0133] In some embodiments, Compound I L-tartrate salt Form A is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 12.0, 18.2, or 20.0 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 8;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 16.1 $^\circ\text{C}$ (onset temperature) and an endotherm at about 138.2 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 9;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 5.1 wt% up to 90 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 10.

[0134] In one embodiment, provided is a nicotinamide co-crystal of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione.

[0135] In some embodiments, provided is crystalline nicotinamide co-crystal of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I nicotinamide co-crystal), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 14.8, 25.9, and 27.3 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0136] In some embodiments, Compound I nicotinamide co-crystal is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 7.3, 18.3, or 19.5 $^\circ 2\theta$; or
- ii) a diffractogram substantially as shown in FIG. 11.

[0137] In one embodiment, provided is a succinate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione.

[0138] In some embodiments, provided is crystalline succinate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I succinate salt Form A), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 3.7, 11.2, and 16.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0139] In some embodiments, Compound I succinate salt Form A is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 13.7, 17.7, or 21.6 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 12;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 14.3 $^\circ\text{C}$ (onset temperature) and an endotherm at about 90.9 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 13;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.4 wt% up to 82 $^\circ\text{C}$, and a further weight loss of about 4.0 wt% from 82 $^\circ\text{C}$ up to 160 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 14.

[0140] In one embodiment, provided is a *R*-mandelate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione.

[0141] In some embodiments, provided is crystalline *R*-mandelate salt of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I *R*-mandelate salt Form A), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 8.8, 14.6, and 19.8 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0142] In some embodiments, Compound I *R*-mandelate salt Form A is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 4.4, 7.3, or 21.2 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 15;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 101.3 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 16;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.4 wt% up to 82 $^\circ\text{C}$, and a further weight loss of about 4.0 wt% from 82 $^\circ\text{C}$ up to 160 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 17.

Salts and Forms of Compound II

[0143] As described generally above, the present disclosure provides salts and solid forms (e.g. crystalline or amorphous forms) of the compound, (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (hereinafter "Compound II"). Salts and solid forms (e.g. crystalline or amorphous forms) of Compound II are collectively referred to herein as "forms of Compound II."

[0144] In some embodiments, Compound II is a free base. In some embodiments, Compound II is a salt. In some embodiments, Compound II is a pharmaceutically acceptable salt. In some embodiments, Compound II is a solvate. In some embodiments, Compound II is a hydrate. In some embodiments, Compound I is unsolvated.

Forms of Compound II

Compound II Form B

[0145] In one embodiment, provided is amorphous (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isindolin-2-yl)piperidine-2,6-dione (Compound II Amorphous Form), characterized by an X-ray powder diffractogram substantially as shown in FIG. 18.

[0146] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isindolin-2-yl)piperidine-2,6-dione (Compound II Form B), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.3, 17.8, and 19.0 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0147] In some embodiments, Compound II Form B is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 12.3, 21.5, or 22.0 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 19;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 104.4 $^\circ\text{C}$ (onset temperature), and endotherm at about 135.3 $^\circ\text{C}$ (onset temperature), and an endotherm at about 197.0 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 20;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 19.9 wt% up to 138 $^\circ\text{C}$, and a further weight loss of about 3.7 wt% from 138 $^\circ\text{C}$ up to 196 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 21.

[0148] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isindolin-2-yl)piperidine-2,6-dione (Compound II Form C), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 4.0, 12.0, and 14.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0149] In some embodiments, Compound II Form C is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 9.6, 16.5, or 19.3 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 22;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 205.6 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 23;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 2.1 wt% up to 180 $^\circ\text{C}$; or

vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 24.

[0150] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form D1), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4, 7.9, and 15.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0151] In some embodiments, Compound II Form D1 is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 12.3, 17.9, or 20.6 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 25;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 74.1 $^\circ\text{C}$ (onset temperature), and an endotherm at about 205.3 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 26;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 2.7 wt% up to 85 $^\circ\text{C}$, and a further weight loss of about 5.5 wt% from 85 $^\circ\text{C}$ up to 134 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 27.

[0152] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form D2), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4, 16.2, and 17.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0153] In some embodiments, Compound II Form D2 is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 4.0, 8.1, and 19.2 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 28;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 31.7 $^\circ\text{C}$ (onset temperature), an endotherm at about 79.2 $^\circ\text{C}$ (onset temperature), an endotherm at about 113.8 $^\circ\text{C}$ (onset temperature), an exotherm at about 156.4 $^\circ\text{C}$ (onset temperature), and an endotherm at about 194.6 $^\circ\text{C}$ (onset temperature); or
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 29;

[0154] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form D3), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4, 8.1, and 16.2 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0155] In some embodiments, Compound II Form D3 is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 12.8, 8.0, or 20.7 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 30;

- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 65.9 °C (onset temperature) and an endotherm at about 124.8 °C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 31;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 4.5 wt% up to 117 °C, and a further weight loss of about 0.7 wt% from 117 °C up to 250 °C; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 32.

[0156] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form E), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.1, 7.6, and 15.2 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0157] In some embodiments, Compound II Form E is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 12.2, 13.5, or 21.2 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 33;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 12.6 °C (onset temperature), an endotherm at about 93.4 °C (onset temperature), and an endotherm at about 197.0 °C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 34;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 2.0 wt% up to 80 °C, and a further weight loss of about 7.3 wt% from 80 °C up to 140 °C; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 35.

[0158] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form F), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.2, 18.0, and 19.2 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0159] In some embodiments, Compound II Form F is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 14.1, 17.4, or 20.1 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 36;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 99.4 °C (onset temperature) and an endotherm at about 208.7 °C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 37;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 0.8 wt% up to 95 °C, and a further weight loss of about 14.2 wt% from 95 °C up to 140 °C; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 38.

[0160] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isindolin-2-yl)piperidine-2,6-dione (Compound II Form G), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.3, 18.4, and 19.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0161] In some embodiments, Compound II Form G is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 11.1, 14.3, or 16.9 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 39;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 45.4 $^\circ\text{C}$ (onset temperature), an endotherm at about 105.6 $^\circ\text{C}$ (onset temperature), and an exotherm at about 177.0 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 40;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 6.0 wt% up to 150 $^\circ\text{C}$, and a further weight loss of about 4.3 wt% from 150 $^\circ\text{C}$ up to 250 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 41.

[0162] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isindolin-2-yl)piperidine-2,6-dione (Compound II Form H), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.2, 18.1, and 21.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0163] In some embodiments, Compound II Form H is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 11.0, 14.9, or 18.2 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 42;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 117.9 $^\circ\text{C}$ (onset temperature) and an exotherm at about 179.2 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 43;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.6 wt% up to 115 $^\circ\text{C}$, and a further weight loss of about 8.1 wt% from 115 $^\circ\text{C}$ up to 150 $^\circ\text{C}$;
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 44;
- vii) unit cell parameters: $a = 24.1274$ (6), $\text{\AA}$, $b = 24.1274$ (6) $\text{\AA}$, $c = 13.0308$ (4) $\text{\AA}$, $V = 6569.4$ (4) $\text{\AA}^3$;
- viii) unit cell parameters: $a = 24.2881$ (6), $\text{\AA}$, $b = 24.2881$ (6) $\text{\AA}$, $c = 13.0707$ (4) $\text{\AA}$, $V = 6677.5$ (4) $\text{\AA}^3$; or
- ix) unit cell parameters: $a = 24.2722$ (6), $\text{\AA}$, $b = 24.2711$ (6) $\text{\AA}$, $c = 13.0585$ (4) $\text{\AA}$, $V = 6662.6$ (4) $\text{\AA}^3$.

[0164] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form I), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 15.4, 15.8, and 21.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0165] In some embodiments, Compound II Form I is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 7.0, 20.3, or 21.2 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 48;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 94.1 $^\circ\text{C}$ (onset temperature) and an endotherm at about 186.2 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 49;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 15.9 wt% up to 91 $^\circ\text{C}$, a further weight loss of about 18.1 wt% from 91 $^\circ\text{C}$ up to 179 $^\circ\text{C}$, and a further weight loss of about 7.8 wt% from 179 $^\circ\text{C}$ up to 261 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 50.

[0166] In one embodiment, provided is crystalline (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form J), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 17.2, 17.6, and 20.4 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0167] In some embodiments, Compound II Form J is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 7.2, 18.3, or 19.0 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 51;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 82.3 $^\circ\text{C}$ (onset temperature), an endotherm at about 103.0 $^\circ\text{C}$ (onset temperature), an endotherm at about 127.1 $^\circ\text{C}$ (onset temperature), and an endotherm at about 214.9 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 52;

[0168] In one embodiment, provided is a fumarate salt of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione.

[0169] In some embodiments, provided is crystalline fumarate salt of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II fumarate salt Form B), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 9.9, 19.3, and 20.0 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0170] In some embodiments, Compound II fumarate salt Form B is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 16.5, 18.6, or 23.0 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 53;

- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 19.8 °C (onset temperature), an endotherm at about 118.2 °C (onset temperature), an endotherm at about 181.7 °C (onset temperature), an exotherm at about 195.5 °C (onset temperature), and an endotherm at about 200.7 °C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 54;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.4 wt% up to 165 °C, and a further weight loss of about 7.8 wt% from 165 °C up to 250 °C; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 55.

[0171] In some embodiments, provided is crystalline fumarate salt of (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II fumarate salt Form C), characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 9.6, 17.5, and 18.1 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

[0172] In some embodiments, Compound II fumarate salt Form C is further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 14.9, 19.6, or 21.1 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 56;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 19.7 °C (onset temperature), an endotherm at about 99.2 °C (onset temperature), and an endotherm at about 161.5 °C (onset temperature); or
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 57.

Administration, Pharmaceutical Compositions

[0173] In some embodiments, provided is a composition comprising a salt or solid form of 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I) or (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II), as described herein.

[0174] In one embodiment, provided is a composition comprising a salt or solid form of 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I), or salt or solvate thereof, wherein at least 50% to 99% (e.g., at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%) of Compound I present in a composition is in the designated salt, solid form, crystalline form, or crystalline salt form.

[0175] In one embodiment, provided is a composition comprising a salt or solid form of (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II), or salt or solvate thereof, wherein at least 50% to 99% (e.g., at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%) of

Compound II present in a composition is in the designated salt, solid form, crystalline form, or crystalline salt form.

[0176] In one embodiment, provided is a composition comprising a crystalline form of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II Form C), or salt or solvate thereof, wherein at least 50% to 99% (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%) of Compound II present in a composition is in the designated salt, solid form, crystalline form, or crystalline salt form.

[0177] In some embodiments, the composition is a pharmaceutical composition which further comprises a pharmaceutically acceptable excipient.

[0178] Administration of the disclosed compounds and pharmaceutical compositions can be accomplished via any mode of administration for therapeutic agents. These modes include systemic or local administration such as oral, nasal, parenteral, transdermal, subcutaneous, vaginal, buccal, rectal or topical administration modes.

[0179] Depending on the intended mode of administration, the disclosed compositions can be in solid, semi-solid or liquid dosage form, such as, for example, injectables, tablets, suppositories, pills, time-release capsules, elixirs, tinctures, emulsions, syrups, powders, liquids, suspensions, or the like, sometimes in unit dosages and consistent with conventional pharmaceutical practices. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous or intramuscular form, and all using forms well known to those skilled in the pharmaceutical arts.

[0180] Illustrative pharmaceutical compositions are tablets and gelatin capsules comprising a compound of the disclosure and a pharmaceutically acceptable carrier, such as a) a diluent, *e.g.*, purified water, triglyceride oils, such as hydrogenated or partially hydrogenated vegetable oil, or mixtures thereof, corn oil, olive oil, sunflower oil, safflower oil, fish oils, such as EPA or DHA, or their esters or triglycerides or mixtures thereof, omega-3 fatty acids or derivatives thereof, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose, sodium, saccharin, glucose and/or glycine; b) a lubricant, *e.g.*, silica, talcum, stearic acid, its magnesium or calcium salt, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and/or polyethylene glycol; for tablets also; c) a binder, *e.g.*, magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, magnesium carbonate, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, waxes, and/or polyvinylpyrrolidone, if desired; d) a disintegrant, *e.g.*, starches, agar, methyl cellulose, bentonite, xanthan gum, algic acid or its sodium salt, or effervescent mixtures; e) absorbent, colorant, flavorant and sweetener; f) an emulsifier or dispersing agent, such as Tween 80, Labrasol, HPMC, DOSS, caproyl 90, labrafac, labrafil, peceol, transcitol, capmul MCM, capmul PG-12, captex 355, gelucire, vitamin E TGPS or other acceptable emulsifier; and/or g) an agent that enhances absorption of the compound such as cyclodextrin, hydroxypropyl-cyclodextrin, PEG400, PEG200.

[0181] Liquid, particularly injectable, compositions can, for example, be prepared by dissolution, dispersion, etc. For example, the disclosed compound is dissolved in or mixed with a pharmaceutically acceptable solvent such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form an injectable isotonic solution or suspension. Proteins such as albumin, chylomicron particles, or serum proteins can be used to solubilize the disclosed compounds.

[0182] The disclosed compounds can be also formulated as a suppository that can be prepared from fatty emulsions or suspensions; using polyalkylene glycols such as propylene glycol, as the carrier.

[0183] The disclosed compounds can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine or phosphatidylcholines.

[0184] In some embodiments, a film of lipid components is hydrated with an aqueous solution of drug to a form lipid layer encapsulating the drug, as described in U.S. Pat. No. 5,262,564, which is hereby incorporated by reference in its entirety.

[0185] Disclosed compounds can also be delivered by the use of monoclonal antibodies as individual carriers to which the disclosed compounds are coupled. The disclosed compounds can also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxyethylaspanamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the disclosed compounds can be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates, and cross-linked or amphipathic block copolymers of hydrogels. In one embodiment, disclosed compounds are not covalently bound to a polymer, e.g., a polycarboxylic acid polymer, or a polyacrylate.

[0186] Parental injectable administration is generally used for subcutaneous, intramuscular or intravenous injections and infusions. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions or solid forms suitable for dissolving in liquid prior to injection.

[0187] Another aspect of the disclosure is directed to pharmaceutical compositions comprising Compound I or II or any salt or form thereof, and a pharmaceutically acceptable carrier. The pharmaceutical acceptable carrier may further include an excipient, diluent, or surfactant.

[0188] Compositions can be prepared according to conventional mixing, granulating or coating methods, respectively, and the present pharmaceutical compositions can contain from about 0.1% to about 99%, from about 5% to about 90%, or from about 1% to about 20% of the disclosed compound by weight or volume.

[0189] In one embodiment, the disclosure provides a kit comprising two or more separate pharmaceutical compositions, at least one of which contains a compound of the present disclosure. In one embodiment, the kit comprises means for separately retaining said compositions, such as a container, divided bottle, or

divided foil packet. An example of such a kit is a blister pack, as typically used for the packaging of tablets, capsules and the like.

[0190] The kit of the disclosure may be used for administering different dosage forms, for example, oral and parenteral, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit of the disclosure typically comprises directions for administration.

[0191] Pharmaceutical dosage forms of a compound of this disclosure may be manufactured by any of the methods well-known in the art, such as, for example, by conventional mixing, sieving, dissolving, melting, granulating, dragee-making, tableting, suspending, extruding, spray-drying, levigating, emulsifying, (nano-/micro-) encapsulating, entrapping, or lyophilization processes. As noted above, the compositions of this disclosure can include one or more physiologically acceptable inactive ingredients that facilitate processing of active molecules into preparations for pharmaceutical use.

[0192] As noted above, the compositions are comprised of, in general, a compound of this disclosure in combination with at least one pharmaceutically acceptable excipient. Acceptable excipients are non-toxic, aid administration, and do not adversely affect the therapeutic benefit of the claimed compounds. Such excipient may be any solid, liquid, semi-solid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[0193] Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semi-solid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. In some embodiments, liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

[0194] Compressed gases may be used to disperse a compound of this disclosure in an aerosol form. Inert gases suitable for this purpose are nitrogen, carbon dioxide, etc. Other suitable pharmaceutical excipients and their formulations are described in Remington's Pharmaceutical Sciences, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

[0195] The compositions of this disclosure may, if desired, be presented in a pack or dispenser device containing one or more unit dosage forms containing the active ingredient. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack, or glass, and rubber stoppers such as in vials. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of this disclosure that can be formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

[0196] The amount of the compound in a formulation can vary within the full range employed by those skilled in the art. Typically, the formulation will contain, on a weight percent (wt %) basis, from about 0.01-

99.99 wt % of a compound of this disclosure based on the total formulation, with the balance being one or more suitable pharmaceutical excipients. In one embodiment, the compound is present at a level of about 1-80 wt %. Representative pharmaceutical formulations are described below.

Formulation Examples

[0197] The following are representative pharmaceutical formulations containing a compound of this disclosure.

Formulation Example 1 - Tablet formulation

[0198] The following ingredients are mixed intimately and pressed into single scored tablets.

Ingredient	Quantity per tablet, mg
compound of this disclosure	400
Cornstarch	50
croscarmellose sodium	25
Lactose	120
magnesium stearate	5

Formulation Example 2 - Capsule formulation

[0199] The following ingredients are mixed intimately and loaded into a hard-shell gelatin capsule

Ingredient	Quantity per capsule, mg
compound of this disclosure	200
lactose, spray-dried	148
magnesium stearate	2

Formulation Example 3 - Suspension formulation

[0200] The following ingredients are mixed to form a suspension for oral administration.

Ingredient	Amount
compound of this disclosure	1.0 g
fumaric acid	0.5 g
sodium chloride	2.0 g
methyl paraben	0.15 g
propyl paraben	0.05 g
granulated sugar	25.0 g
sorbitol (70% solution)	13.00 g

Veegum K (Vanderbilt Co.)	1.0 g
Flavoring	0.035 mL
Colorings	0.5 mg
distilled water	q.s. to 100 mL

Formulation Example 4 - Injectable formulation

[0201] The following ingredients are mixed to form an injectable formulation.

Ingredient	Amount
compound of this disclosure	0.2 mg-20 mg
sodium acetate buffer solution, 0.4 M	2.0 mL
HCl (1N) or NaOH (1N)	q.s. to suitable pH
water (distilled, sterile)	q.s. to 20 mL

Formulation Example 5 - Suppository Formulation

[0202] A suppository of total weight 2.5 g is prepared by mixing the compound of this disclosure with Witepsol® H-15 (triglycerides of saturated vegetable fatty acid; Riches-Nelson, Inc., New York), and has the following composition:

Ingredient	Amount
Compound of this disclosure	500 mg
Witepsol® H-15	balance

Dosing

[0203] The dosage regimen utilizing the disclosed compound is selected in accordance with a variety of factors including type, species, age, weight, sex, and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal or hepatic function of the patient; and the particular disclosed compound employed. A physician or veterinarian of ordinary skill in the art can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

[0204] Effective dosage amounts of the disclosed compounds, when used for the indicated effects, range from about 0.5 mg to about 5000 mg of the disclosed compound as needed to treat the condition. Compositions for *in vivo* or *in vitro* use can contain about 0.5, 5, 20, 50, 75, 100, 150, 250, 500, 750, 1000, 1250, 2500, 3500, or 5000 mg of the disclosed compound, or, in a range of from one amount to another amount in the list of doses. In one embodiment, the compositions are in the form of a tablet that can be scored.

[0205] The following synthetic and biological examples are offered to illustrate this disclosure and are not to be construed in any way as limiting the scope of this disclosure. Unless otherwise stated, all temperatures are in degrees Celsius.

Methods of Treatment

[0206] Provided herein is a method for modulating cereblon activity, which method comprises contacting cereblon with an effective amount of a salt or solid form of Compound I or Compound II, or a pharmaceutically acceptable salt, solvate, stereoisomer, or tautomer thereof, under conditions wherein cereblon is modulated.

[0207] Also provided herein is a method for degrading IKZF2, which method comprises contacting IKZF2 with an effective amount of a salt or solid form of Compound I or Compound II, or a pharmaceutically acceptable salt, solvate, stereoisomer, and/or tautomer thereof, under conditions wherein IKZF2 is degraded.

[0208] Also provided herein is a method to degrade IKZF2 in a subject, which method comprises administering to said subject an effective amount of a salt or solid form of Compound I or Compound II, or a pharmaceutically acceptable salt, solvate, stereoisomer, and/or tautomer thereof.

[0209] Also provided herein is a method to treat cancer in a subject in need thereof, which method comprises selecting a subject whose cancer is mediated at least in part by IKZF2 and administering to said subject an effective amount of a salt or solid form of Compound I or Compound II, or a pharmaceutically acceptable salt, solvate, stereoisomer, and/or tautomer thereof.

[0210] Also provided herein is a method to treat cancer in a subject in need thereof, which method comprises selecting a subject whose cancer is mediated at least in part by IKZF2 and administering to said subject an effective amount of a pharmaceutical composition comprising a therapeutically effective amount of a salt or solid form of Compound I or Compound II and a pharmaceutically acceptable excipient.

[0211] In some embodiments, the cancer is non-small cell lung cancer (NSCLC), melanoma, triple-negative breast cancer (TNBC), nasopharyngeal cancer (NPC), microsatellite stable colorectal cancer (mssCRC), thymoma, carcinoid, acute myelogenous leukemia, or gastrointestinal stromal tumor (GIST).

[0212] This disclosure features methods for treating a subject (e.g., a human) having a disease, disorder, or condition modulated by IKZF2. Non-limiting examples of IKZF2 dependent diseases or disorders include proliferative diseases or disorders which may be non-cancerous or cancerous.

[0213] Examples of non-cancerous conditions or disorders include, but are not limited to, rheumatoid arthritis; inflammation; autoimmune disease; lymphoproliferative conditions; acromegaly; rheumatoid spondylitis; osteoarthritis; gout, other arthritic conditions; sepsis; septic shock; endotoxic shock; gram-negative sepsis; toxic shock syndrome; asthma; adult respiratory distress syndrome; chronic obstructive pulmonary disease; chronic pulmonary inflammation; inflammatory bowel disease; Crohn's disease; psoriasis; eczema; ulcerative colitis; pancreatic fibrosis; hepatic fibrosis; acute and chronic renal disease; irritable bowel syndrome; pyresis; restenosis; cerebral malaria; stroke and ischemic injury; neural trauma;

Alzheimer's disease; Huntington's disease; Parkinson's disease; acute and chronic pain; allergic rhinitis; allergic conjunctivitis; chronic heart failure; acute coronary syndrome; cachexia; malaria; leprosy; leishmaniasis; Lyme disease; Reiter's syndrome; acute synovitis; muscle degeneration, bursitis; tendonitis; tenosynovitis; herniated, ruptures, or prolapsed intervertebral disk syndrome; osteopetrosis; thrombosis; restenosis; silicosis; pulmonary sarcoidosis; bone resorption diseases, such as osteoporosis; graft-versus-host reaction; Multiple Sclerosis; lupus; fibromyalgia; AIDS and other viral diseases such as Herpes Zoster, Herpes Simplex I or II, influenza virus and cytomegalovirus; and diabetes mellitus.

[0214] In certain embodiments, the chemical entities or compositions described herein are useful in the treatment of cancers and other proliferative disorders, including, but not limited to breast cancer, cervical cancer, colon and rectal cancer, leukemia, lung cancer, melanoma, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, and gastric cancer. In certain embodiments, compounds or compositions described herein are active against solid tumors.

[0215] In certain embodiments, the chemical entities or compositions described herein are useful for the treatment of cancer (including, but not limited to, glioblastoma, retinoblastoma, breast cancer, cervical cancer, colon and rectal cancer, leukemia, lymphoma, lung cancer (including, but not limited to small cell lung cancer), melanoma and/or skin cancer, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer and gastric cancer, bladder cancer, uterine cancer, kidney cancer, testicular cancer, stomach cancer, brain cancer, liver cancer, or esophageal cancer).

[0216] In some embodiments, examples of cancers include, but are not limited to, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, anorectal cancer, cancer of the anal canal, appendix cancer, childhood cerebellar astrocytoma, childhood cerebral astrocytoma, basal cell carcinoma, skin cancer (non-melanoma), biliary cancer, extrahepatic bile duct cancer, intrahepatic bile duct cancer, bladder cancer, urinary bladder cancer, bone and joint cancer, osteosarcoma and malignant fibrous histiocytoma, brain cancer, brain tumor, brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas/carcinoids, carcinoid tumor, gastrointestinal, nervous system cancer, nervous system lymphoma, central nervous system cancer, central nervous system lymphoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, cutaneous T-cell lymphoma, lymphoid neoplasm, mycosis fungoides, Sezary Syndrome, endometrial cancer, esophageal cancer, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor, ovarian germ cell tumor, gestational trophoblastic tumor glioma, head and neck cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, ocular cancer, islet cell tumors (endocrine pancreas), Kaposi Sarcoma, kidney cancer, renal cancer, kidney cancer, laryngeal cancer, acute

lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, lip and oral cavity cancer, liver cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, AIDS-related lymphoma, non-Hodgkin lymphoma, primary central nervous system lymphoma, Waldenstrom macroglobulinemia, medulloblastoma, melanoma, intraocular (eye) melanoma, merkel cell carcinoma, mesothelioma malignant, mesothelioma, metastatic squamous neck cancer, mouth cancer, cancer of the tongue, multiple endocrine neoplasia syndrome, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, chronic myelogenous leukemia, acute myeloid leukemia, multiple myeloma, chronic myeloproliferative disorders, nasopharyngeal cancer, neuroblastoma, oral cancer, oral cavity cancer, oropharyngeal cancer, ovarian cancer, ovarian epithelial cancer, ovarian low malignant potential tumor, pancreatic cancer, islet cell pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, prostate cancer, rectal cancer, renal pelvis and ureter, transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, Ewing family of sarcoma tumors, Kaposi Sarcoma, soft tissue sarcoma, uterine cancer, uterine sarcoma, skin cancer (non-melanoma), skin cancer (melanoma), Merkel cell skin carcinoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach (gastric) cancer, supratentorial primitive neuroectodermal tumors, testicular cancer, throat cancer, thymoma, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter and other urinary organs, gestational trophoblastic tumor, urethral cancer, endometrial uterine cancer, uterine sarcoma, uterine corpus cancer, vaginal cancer, vulvar cancer, and Wilms' Tumor.

[0217] In certain embodiments, the chemical entities described herein are useful for the treatment of cancer (including, but not limited to, glioblastoma, retinoblastoma, breast cancer, cervical cancer, colon and rectal cancer, leukemia, lymphoma, lung cancer (including, but not limited to small cell lung cancer), melanoma and/or skin cancer, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer and gastric cancer, bladder cancer, uterine cancer, kidney cancer, testicular cancer, stomach cancer, brain cancer, liver cancer, or esophageal cancer) and/or any other cancer described herein.

[0218] In certain embodiments, the chemical entities described herein are useful in the treatment of cancers and other proliferative disorders, including, but not limited to breast cancer, cervical cancer, colon and rectal cancer, leukemia, lung cancer, melanoma, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, and gastric cancer. In certain embodiments, the compounds are active against solid tumors.

[0219] In certain embodiments, the chemical entities and compositions described herein are useful in treating IKZF2 dependent diseases or disorders such as liposarcoma, glioblastoma, bladder cancer, adrenocortical cancer, multiple myeloma, colorectal cancer, non-small cell lung cancer, Human Papilloma Virus-associated cervical, oropharyngeal, penis, anal, thyroid, or vaginal cancer or Epstein-Barr Virus-

associated nasopharyngeal carcinoma, gastric cancer, rectal cancer, thyroid cancer, Hodgkin lymphoma or diffuse large B-cell lymphoma. The cancer may be selected from prostate cancer, breast carcinoma, lymphomas, leukemia, myeloma, bladder carcinoma, colon cancer, cutaneous melanoma, hepatocellular carcinoma, endometrial cancer, ovarian cancer, cervical cancer, lung cancer, renal cancer, glioblastoma multiform, glioma, thyroid cancer, parathyroid tumor, nasopharyngeal cancer, tongue cancer, pancreatic cancer, esophageal cancer, cholangiocarcinoma, gastric cancer, soft tissue sarcomas, rhabdomyosarcoma (RMS), synovial sarcoma, osteosarcoma, rhabdoid cancers, cancer for which the immune response is deficient, an immunogenic cancer, and Ewing's sarcoma. In one embodiment, the IKZF2-dependent disease or disorder is a disease or disorder is selected from non-small cell lung cancer (NSCLC), melanoma, triple-negative breast cancer (TNBC), nasopharyngeal cancer (NPC), microsatellite stable colorectal cancer (mssCRC), thymoma, carcinoid, and gastrointestinal stromal tumor (GIST). In another embodiment, the cancer is selected from non-small cell lung cancer (NSCLC), melanoma, triple-negative breast cancer (TNBC), nasopharyngeal cancer (NPC), microsatellite stable colorectal cancer (mssCRC), thymoma, carcinoid, acute myelogenous leukemia, and gastrointestinal stromal tumor (GIST). In another embodiment, the IKZF2-dependent disease or disorder is a disease or disorder is selected from non-small cell lung cancer (NSCLC), melanoma, triple-negative breast cancer (TNBC), nasopharyngeal cancer (NPC), and microsatellite stable colorectal cancer (mssCRC).

[0220] The chemical entities of the disclosure can be administered in effective amounts to treat or prevent a disorder and/or prevent the development thereof in subjects.

[0221] In general, methods of using the chemical entities of the present application comprise administering to a subject in need thereof a therapeutically effective amount of a chemical entity as described herein.

[0222] In certain embodiments, chemical entities as described herein are useful in the treatment of proliferative diseases (e.g., cancer, benign neoplasms, inflammatory disease, and autoimmune diseases). In certain embodiments, according to the methods of treatment of the present application, levels of cell proteins of interest, e.g., pathogenic and oncogenic proteins are modulated, or their growth is inhibited or the proteins are degraded by contacting said cells with a chemical entity or composition, as described herein. In other embodiments, the chemical entities are useful in treating cancer.

[0223] Thus, in another aspect of the application, methods for the treatment of cancer are provided comprising administering a therapeutically effective amount of a chemical entity or composition, as described herein, to a subject in need thereof. In certain embodiments, a method for the treatment of cancer is provided comprising administering a therapeutically effective amount of a chemical entity, or a pharmaceutical composition comprising a chemical entity as described herein to a subject in need thereof, in such amounts and for such time as is necessary to achieve the desired result. In some embodiments, the chemical entities of present application are administered orally or intravenously. In certain embodiments of the present application a "therapeutically effective amount" of the chemical entities or pharmaceutical composition is that amount effective for killing or inhibiting the growth of tumor cells. The chemical entities

and compositions, according to the method of the present application, may be administered using any amount and any route of administration effective for killing or inhibiting the growth of tumor cells. Thus, the expression “amount effective to kill or inhibit the growth of tumor cells,” as used herein, refers to a sufficient amount of agent to kill or inhibit the growth of tumor cells. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular anticancer agent, its mode of administration, and the like. In certain embodiments of the present application a “therapeutically effective amount” of the chemical entities or pharmaceutical composition described herein is that amount effective for reducing the levels of target proteins. In certain embodiments of the present application a “therapeutically effective amount” of the chemical entities or pharmaceutical composition is that amount effective to kill or inhibit the growth of skin cells.

[0224] In certain embodiments, the method involves the administration of a therapeutically effective amount of the chemical entities or a pharmaceutically acceptable derivative thereof to a subject (including, but not limited to a human or other mammal in need of it.

[0225] Additionally, the present application provides pharmaceutically acceptable derivatives of the chemical entities, and methods of treating a subject using these compounds, pharmaceutical compositions thereof, or either of these in combination with one or more additional therapeutic agents.

[0226] Another aspect of the application relates to a method of treating or lessening the severity of a disease or condition associated with a proliferation disorder in a patient, said method comprising a step of administering to said patient, a chemical entity or a composition comprising said chemical entity as described herein.

[0227] It will be appreciated that the chemical entities and compositions, according to the method of the present application, may be administered using any amount and any route of administration effective for the treatment of cancer and/or disorders associated with cell hyperproliferation. For example, when using the chemical entities for the treatment of cancer, the expression “effective amount” as used herein, refers to a sufficient amount of agent to inhibit cell proliferation, or refers to a sufficient amount to reduce the effects of cancer. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the diseases, the particular anticancer agent, its mode of administration, and the like.

[0228] The present application provides methods for the treatment of a proliferative disorder in a subject in need thereof by administering to a subject in need of such treatment, a therapeutically effective amount of a chemical entity of the present application, or a pharmaceutically acceptable salt, solvate, stereoisomer, and/or tautomer thereof. The proliferative disorder can be cancer or a precancerous condition. The present application further provides the use of a chemical entity of the present application, or a pharmaceutically acceptable salt, salt, solvate, stereoisomer, and/or tautomer thereof, for the preparation of a medicament useful for the treatment of a proliferative disorder.

[0229] The present application also provides methods of protecting against a proliferative disorder in a subject in need thereof by administering a therapeutically effective amount of chemical entities of the present application, or a pharmaceutically acceptable salt, salt, solvate, stereoisomer, and/or tautomer thereof, to a subject in need of such treatment. The proliferative disorder can be cancer or a precancerous condition. The present application also provides the use of chemical entities of the present application, or a pharmaceutically acceptable salt, salt, solvate, stereoisomer, and/or tautomer thereof, for the preparation of a medicament useful for the prevention of a proliferative disorder.

[0230] As used herein, the term “proliferative disorder” refers to conditions in which unregulated or abnormal growth, or both, of cells can lead to the development of an unwanted condition or disease, which may or may not be cancerous. Exemplary proliferative disorders of the application encompass a variety of conditions wherein cell division is deregulated. Exemplary proliferative disorder include, but are not limited to, neoplasms, benign tumors, malignant tumors, pre-cancerous conditions, in situ tumors, encapsulated tumors, metastatic tumors, liquid tumors, solid tumors, immunological tumors, hematological tumors, cancers, carcinomas, leukemias, lymphomas, sarcomas, and rapidly dividing cells. The term “rapidly dividing cell” as used herein is defined as any cell that divides at a rate that exceeds or is greater than what is expected or observed among neighboring or juxtaposed cells within the same tissue. A proliferative disorder includes a precancer or a precancerous condition. A proliferative disorder includes cancer. Preferably, the methods provided herein are used to treat or alleviate a symptom of cancer. The term “cancer” includes solid tumors, as well as hematologic tumors and/or malignancies. A “precancer cell” or “precancerous cell” is a cell manifesting a proliferative disorder that is a precancer or a precancerous condition. A “cancer cell” or “cancerous cell” is a cell manifesting a proliferative disorder that is a cancer. Any reproducible means of measurement may be used to identify cancer cells or precancerous cells. Cancer cells or precancerous cells can be identified by histological typing or grading of a tissue sample (e.g., a biopsy sample). Cancer cells or precancerous cells can be identified through the use of appropriate molecular markers.

[0231] A “proliferative disorder of the hematologic system” is a proliferative disorder involving cells of the hematologic system. A proliferative disorder of the hematologic system can include lymphoma, leukemia, myeloid neoplasms, mast cell neoplasms, myelodysplasia, benign monoclonal gammopathy, lymphomatoid granulomatosis, lymphomatoid papulosis, polycythemia vera, chronic myelocytic leukemia, agnogenic myeloid metaplasia, and essential thrombocythemia. A proliferative disorder of the hematologic system can include hyperplasia, dysplasia, and metaplasia of cells of the hematologic system. Preferably, compositions of the present application may be used to treat a cancer selected from the group consisting of a hematologic cancer of the present application or a hematologic proliferative disorder of the present application. A hematologic cancer of the present application can include multiple myeloma, lymphoma (including Hodgkin's lymphoma, non-Hodgkin's lymphoma, childhood lymphomas, and lymphomas of lymphocytic and cutaneous origin), leukemia (including childhood leukemia, hairy-cell leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, chronic myelogenous leukemia, and mast cell leukemia), myeloid neoplasms and mast cell neoplasms.

[0232] A “proliferative disorder of the lung” is a proliferative disorder involving cells of the lung. Proliferative disorders of the lung can include all forms of proliferative disorders affecting lung cells. Proliferative disorders of the lung can include lung cancer, a precancer or precancerous condition of the lung, benign growths or lesions of the lung, and malignant growths or lesions of the lung, and metastatic lesions in tissue and organs in the body other than the lung. Preferably, compositions of the present application may be used to treat lung cancer or proliferative disorders of the lung. Lung cancer can include all forms of cancer of the lung. Lung cancer can include malignant lung neoplasms, carcinoma in situ, typical carcinoid tumors, and atypical carcinoid tumors. Lung cancer can include small cell lung cancer (“SCLC”), non-small cell lung cancer (“NSCLC”), squamous cell carcinoma, adenocarcinoma, small cell carcinoma, large cell carcinoma, adenosquamous cell carcinoma, and mesothelioma. Lung cancer can include “scar carcinoma”, bronchioalveolar carcinoma, giant cell carcinoma, spindle cell carcinoma, and large cell neuroendocrine carcinoma. Lung cancer can include lung neoplasms having histologic and ultrastructural heterogeneity (e.g., mixed cell types).

[0233] Proliferative disorders of the lung can include all forms of proliferative disorders affecting lung cells. Proliferative disorders of the lung can include lung cancer, precancerous conditions of the lung. Proliferative disorders of the lung can include hyperplasia, metaplasia, and dysplasia of the lung. Proliferative disorders of the lung can include asbestos-induced hyperplasia, squamous metaplasia, and benign reactive mesothelial metaplasia. Proliferative disorders of the lung can include replacement of columnar epithelium with stratified squamous epithelium, and mucosal dysplasia. Individuals exposed to inhaled injurious environmental agents such as cigarette smoke and asbestos may be at increased risk for developing proliferative disorders of the lung. Prior lung diseases that may predispose individuals to development of proliferative disorders of the lung can include chronic interstitial lung disease, necrotizing pulmonary disease, scleroderma, rheumatoid disease, sarcoidosis, interstitial pneumonitis, tuberculosis, repeated pneumonias, idiopathic pulmonary fibrosis, granulomata, asbestosis, fibrosing alveolitis, and Hodgkin's disease.

[0234] A “proliferative disorder of the colon” is a proliferative disorder involving cells of the colon. Preferably, the proliferative disorder of the colon is colon cancer. Preferably, compositions of the present application may be used to treat colon cancer or proliferative disorders of the colon. Colon cancer can include all forms of cancer of the colon. Colon cancer can include sporadic and hereditary colon cancers. Colon cancer can include malignant colon neoplasms, carcinoma in situ, typical carcinoid tumors, and atypical carcinoid tumors. Colon cancer can include adenocarcinoma, squamous cell carcinoma, and adenosquamous cell carcinoma. Colon cancer can be associated with a hereditary syndrome selected from the group consisting of hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, Gardner's syndrome, Peutz-Jeghers syndrome, Turcot's syndrome and juvenile polyposis. Colon cancer can be caused by a hereditary syndrome selected from the group consisting of hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, Gardner's syndrome, Peutz-Jeghers syndrome, Turcot's syndrome and juvenile polyposis.

[0235] Proliferative disorders of the colon can include all forms of proliferative disorders affecting colon cells. Proliferative disorders of the colon can include colon cancer, precancerous conditions of the colon, adenomatous polyps of the colon and metachronous lesions of the colon. A proliferative disorder of the colon can include adenoma. Proliferative disorders of the colon can be characterized by hyperplasia, metaplasia, and dysplasia of the colon. Prior colon diseases that may predispose individuals to development of proliferative disorders of the colon can include prior colon cancer. Current diseases that may predispose individuals to development of proliferative disorders of the colon can include Crohn's disease and ulcerative colitis. A proliferative disorder of the colon can be associated with a mutation in a gene selected from the group consisting of p53, ras, FAP and DCC. An individual can have an elevated risk of developing a proliferative disorder of the colon due to the presence of a mutation in a gene selected from the group consisting of p53, ras, FAP and DCC.

[0236] A "proliferative disorder of the pancreas" is a proliferative disorder involving cells of the pancreas. Proliferative disorders of the pancreas can include all forms of proliferative disorders affecting pancreatic cells. Proliferative disorders of the pancreas can include pancreas cancer, a precancer or precancerous condition of the pancreas, hyperplasia of the pancreas, and dysplasia of the pancreas, benign growths or lesions of the pancreas, and malignant growths or lesions of the pancreas, and metastatic lesions in tissue and organs in the body other than the pancreas. Pancreatic cancer includes all forms of cancer of the pancreas. Pancreatic cancer can include ductal adenocarcinoma, adenosquamous carcinoma, pleomorphic giant cell carcinoma, mucinous adenocarcinoma, osteoclast-like giant cell carcinoma, mucinous cystadenocarcinoma, acinar carcinoma, unclassified large cell carcinoma, small cell carcinoma, pancreatoblastoma, papillary neoplasm, mucinous cystadenoma, papillary cystic neoplasm, and serous cystadenoma. Pancreatic cancer can also include pancreatic neoplasms having histologic and ultrastructural heterogeneity (e.g., mixed cell types).

[0237] A "proliferative disorder of the prostate" is a proliferative disorder involving cells of the prostate. Proliferative disorders of the prostate can include all forms of proliferative disorders affecting prostate cells. Proliferative disorders of the prostate can include prostate cancer, a precancer or precancerous condition of the prostate, benign growths or lesions of the prostate, and malignant growths or lesions of the prostate, and metastatic lesions in tissue and organs in the body other than the prostate. Proliferative disorders of the prostate can include hyperplasia, metaplasia, and dysplasia of the prostate.

[0238] A "proliferative disorder of the skin" is a proliferative disorder involving cells of the skin. Proliferative disorders of the skin can include all forms of proliferative disorders affecting skin cells. Proliferative disorders of the skin can include a precancer or precancerous condition of the skin, benign growths or lesions of the skin, melanoma, malignant melanoma and other malignant growths or lesions of the skin, and metastatic lesions in tissue and organs in the body other than the skin. Proliferative disorders of the skin can include hyperplasia, metaplasia, and dysplasia of the skin.

[0239] A “proliferative disorder of the ovary” is a proliferative disorder involving cells of the ovary. Proliferative disorders of the ovary can include all forms of proliferative disorders affecting cells of the ovary. Proliferative disorders of the ovary can include a precancer or precancerous condition of the ovary, benign growths or lesions of the ovary, ovarian cancer, malignant growths or lesions of the ovary, and metastatic lesions in tissue and organs in the body other than the ovary. Proliferative disorders of the skin can include hyperplasia, metaplasia, and dysplasia of cells of the ovary.

[0240] A “proliferative disorder of the breast” is a proliferative disorder involving cells of the breast. Proliferative disorders of the breast can include all forms of proliferative disorders affecting breast cells. Proliferative disorders of the breast can include breast cancer, a precancer or precancerous condition of the breast, benign growths or lesions of the breast, and malignant growths or lesions of the breast, and metastatic lesions in tissue and organs in the body other than the breast. Proliferative disorders of the breast can include hyperplasia, metaplasia, and dysplasia of the breast.

[0241] A cancer that is to be treated can be staged according to the American Joint Committee on Cancer (AJCC) TNM classification system, where the tumor (T) has been assigned a stage of TX, T1, T1mic, T1a, T1b, T1c, T2, T3, T4, T4a, T4b, T4c, or T4d; and where the regional lymph nodes (N) have been assigned a stage of NX, N0, N1, N2, N2a, N2b, N3, N3a, N3b, or N3c; and where distant metastasis (M) can be assigned a stage of MX, M0, or M1. A cancer that is to be treated can be staged according to an American Joint Committee on Cancer (AJCC) classification as Stage I, Stage IIA, Stage IIB, Stage IIIA, Stage IIIB, Stage IIIC, or Stage IV. A cancer that is to be treated can be assigned a grade according to an AJCC classification as Grade GX (e.g., grade cannot be assessed), Grade 1, Grade 2, Grade 3 or Grade 4. A cancer that is to be treated can be staged according to an AJCC pathologic classification (pN) of pNX, pN0, pN0 (I-), pN0 (I+), pN0 (mol-), pN0 (mol+), pN1, pN1(mi), pN1a, pN1b, pN1c, pN2, pN2a, pN2b, pN3, pN3a, pN3b, or pN3c.

[0242] A cancer that is to be treated can include a tumor that has been determined to be less than or equal to about 2 centimeters in diameter. A cancer that is to be treated can include a tumor that has been determined to be from about 2 to about 5 centimeters in diameter. A cancer that is to be treated can include a tumor that has been determined to be greater than or equal to about 3 centimeters in diameter. A cancer that is to be treated can include a tumor that has been determined to be greater than 5 centimeters in diameter. A cancer that is to be treated can be classified by microscopic appearance as well differentiated, moderately differentiated, poorly differentiated, or undifferentiated. A cancer that is to be treated can be classified by microscopic appearance with respect to mitosis count (e.g., amount of cell division) or nuclear pleiomorphism (e.g., change in cells). A cancer that is to be treated can be classified by microscopic appearance as being associated with areas of necrosis (e.g., areas of dying or degenerating cells). A cancer that is to be treated can be classified as having an abnormal karyotype, having an abnormal number of chromosomes, or having one or more chromosomes that are abnormal in appearance. A cancer that is to be treated can be classified as being aneuploid, triploid, tetraploid, or as having an altered ploidy. A cancer that

is to be treated can be classified as having a chromosomal translocation, or a deletion or duplication of an entire chromosome, or a region of deletion, duplication or amplification of a portion of a chromosome.

[0243] A cancer that is to be treated can be evaluated by DNA cytometry, flow cytometry, or image cytometry. A cancer that is to be treated can be typed as having 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of cells in the synthesis stage of cell division (e.g., in S phase of cell division). A cancer that is to be treated can be typed as having a low S-phase fraction or a high S-phase fraction.

[0244] As used herein, a “normal cell” is a cell that cannot be classified as part of a “proliferative disorder”. A normal cell lacks unregulated or abnormal growth, or both, that can lead to the development of an unwanted condition or disease. Preferably, a normal cell possesses normally functioning cell cycle checkpoint control mechanisms.

[0245] One skilled in the art may refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. (2005); Sambrook et al., *Molecular Cloning, A Laboratory Manual* (3rd edition), Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2000); Coligan et al., *Current Protocols in Immunology*, John Wiley & Sons, N.Y.; Erma et al., *Current Protocols in Pharmacology*, John Wiley & Sons, N.Y.; Fingl et al., *The Pharmacological Basis of Therapeutics* (1975), Remington's *Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 18th edition (1990). These texts can, of course, also be referred to in making or using an aspect of the application.

[0246] In certain embodiments, chemical entities of the application are useful in the treatment of proliferative diseases (e.g., cancer, benign neoplasms, inflammatory disease, and autoimmune diseases). In certain embodiments, according to the methods of treatment of the present application, levels of cell proteins of interest, e.g., pathogenic and oncogenic proteins are modulated, or their growth is inhibited by contacting said cells with a compound or composition, as described herein. In other embodiments, the chemical entities are useful in treating cancer.

[0247] In certain embodiments, the method involves the administration of a therapeutically effective amount of the chemical entities or a pharmaceutically acceptable derivative thereof to a subject (including, but not limited to a human or animal) in need of it.

[0248] Additionally, the present application provides pharmaceutically acceptable derivatives of the chemical entities, and methods of treating a subject using these compounds, pharmaceutical compositions thereof, or either of these in combination with one or more additional therapeutic agents.

[0249] For example, other therapies or anticancer agents that may be used in combination with the chemical entities disclosed herein including surgery, radiotherapy, endocrine therapy, biologic response modifiers (interferons, interleukins, and tumor necrosis factor (TNF), to name a few), hyperthermia and cryotherapy, agents to attenuate any adverse effects (e.g., antiemetics), and other approved chemotherapeutic drugs, including, but not limited to, alkylating drugs (mechlorethamine, chlorambucil, cyclophosphamide,

melphalan, ifosfamide), antimetabolites (methotrexate), purine antagonists and pyrimidine antagonists (6-mercaptopurine, 5-fluorouracil, cytarabine, gemcitabine), spindle poisons (vinblastine, vincristine, vinorelbine, paclitaxel), podophyllotoxins (etoposide, irinotecan, topotecan), antibiotics (doxorubicin, bleomycin, mitomycin), nitrosoureas (carmustine, lomustine), inorganic ions (cisplatin, carboplatin), enzymes (asparaginase), and hormones (tamoxifen, leuprolide, flutamide, and megestrol), to name a few. For a more comprehensive discussion of overview of cancer therapy see The Merck Manual, Twentieth Ed. 2020, the entire contents of which are hereby incorporated by reference. See also the National Cancer Institute (NCI) website (www.nci.nih.gov) and the Food and Drug Administration (FDA) website for a list of the FDA approved oncology drugs (www.fda.gov/cder/cancer/druglistframe).

[0250] In certain embodiments, the pharmaceutical compositions comprising the chemical entities disclosed herein further comprise one or more additional therapeutically active ingredients (e.g., chemotherapeutic and/or palliative). For purposes of the application, the term “palliative” refers to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, palliative treatment encompasses painkillers, anti-nausea medications and anti-sickness drugs. In addition, chemotherapy, radiotherapy and surgery can all be used palliatively (that is, to reduce symptoms without going for cure; e.g., for shrinking tumors and reducing pressure, bleeding, pain and other symptoms of cancer).

EXAMPLES

[0251] The following examples are included to demonstrate specific embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques to function well in the practice of the disclosure, and thus can be considered to constitute specific modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Instruments and Methods

X-Ray Powder Diffraction (XRPD)

[0252] X-ray powder diffraction (XRPD) analysis was performed using the following instrument and parameters.

X-ray Powder Diffractometer (XRPD)	
Instrument	Bruker D8 Advance
X-ray geometry	Reflection
Detector	LYNXEYE_XE_T(1D mode)
Open angle	2.9° (max)
Radiation	Cu/K-Alpha1 ($\lambda=1.5406 \text{ \AA}$)

X-ray generator power	40 kV, 40 mA
Primary beam path slits	Twin_Primary motorized slit 10.0 mm by sample length; Primary Soller slit 2.5°
Secondary beam path slits	Detector OpticsMount soller slit 2.5°; Twin_Secondary motorized slit 5.2 mm
Scan mode	Continuous scan
Scan type	Locked coupled
Step size	0.02°
Time per step	0.12 second per step
Scan range	3° to 40°
Sample rotation speed	15 rpm
Sample holder	Flat monocrystalline silicon

[0253] Single Crystal X-ray Diffraction (SCXRD)

[0254] Single crystal X-ray diffraction (SCXRD) analysis was performed using the following instrument and parameters.

Single Crystal X-ray Diffractometer (SCXRD)	
Instrument	Bruker D8 Venture
Method 1 (for SCXRD-01 and SCXRD-02)	
Detector	CMOS area detector
Temperature	100(2) K
Radiation	Cu/K-Alpha1 ($\lambda=1.5418 \text{ \AA}$)
X-ray generator power	50 kV, 1.2 mA
Distance from sample to area detector	40 mm
Exposure time	70 seconds
Resolution	0.80 $\text{\AA}$
Method 2 (for SCXRD-03)	
Detector	CMOS area detector
Temperature	100(2) K
Radiation	Cu/K-Alpha1 ($\lambda=1.5418 \text{ \AA}$)

X-ray generator power	50 kV, 1.2 mA
Distance from sample to area detector	40 mm
Exposure time	20 seconds
Resolution	0.80 Å

Microscopy

[0255] Polarized light microscope (PLM) and stereomicroscope images were collected using the following instrument and parameters.

Polarized Light Microscope (PLM)	
Instrument	Leica DM4P
Method	Crossed polarizer, silicone oil added
Stereomicroscope	
Instrument	Leica S9i

Ultra Performance Convergence Chromatography (UPCC)

[0256] Diastereomeric ratios (dr) of samples were measured by ultra performance convergence chromatography (UPCC), using the following instrument and parameters.

Ultra Performance Convergence Chromatography (UPCC) – Method 1	
Instrument	Waters UPCC with UV detector
	Wave length: Absorbance Compensated 255 nm, Compensation reference 310~350 nm
	Column: Daicel Chiralpak IH-3, 4.6×150 mm, 3.0 µm column, PN: 89524
	Detector: UV
	Column temperature: 40 °C
	Flow rate: 2.0 mL/min
	Mobile phase A: CO ₂
	Mobile phase B: 0.1% IBA (Isopropylamine) in IPA (v/v)
	Diluent: ACN
	Injection volume: 5 µL
	ABPR: 1800 psi

	Needle Wash Solvent: MeOH Gradient: <table><tr><td>Time (min)</td><td>Mobile Phase A (%)</td><td>Mobile Phase B (%)</td></tr><tr><td>0.00</td><td>50</td><td>50</td></tr><tr><td>7.00</td><td>50</td><td>50</td></tr></table>	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	0.00	50	50	7.00	50	50	
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)									
0.00	50	50									
7.00	50	50									
Ultra Performance Convergence Chromatography (UPCC) – Method 2											
Instrument	Waters UPCC with UV detector										
UPCC method	Wave length: 220nm Column: Regis (S,S) Whelk-O 1, 150 mm*4.6(mm), 3.5(μm) PN: 1-780122-300 Detector: UV Column temperature: 40°C Flow rate: 2.0 mL/min Mobile phase A: CO ₂ Mobile phase B1: 0.1% IBA (Isopropylamine) in IPA (v/v) Diluent: ACN Injection volume: 5μL ABPR: 1800psi Needle Wash Solvent: MeOH Gradient: <table><tr><td>Time (min)</td><td>Mobile Phase A (%)</td><td>Mobile Phase B (%)</td></tr><tr><td>0.00</td><td>50</td><td>50</td></tr><tr><td>15.00</td><td>50</td><td>50</td></tr></table>		Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	0.00	50	50	15.00	50	50
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)									
0.00	50	50									
15.00	50	50									

High Performance Liquid Chromatography (HPLC)

[0257] Sample purity was measured via High Performance Liquid Chromatography (HPLC) using the following instrument and parameters.

High Performance Liquid Chromatography (HPLC)			
Instrument	Agilent 1260 infinity II Binary Pump		
HPLC method	Wave length: 220 nm		
	Column: Agilent Zorbax Eclipse XDB-C18 4.6mm*150mm 5µm		
	Detector: DAD		
	Column temperature: 40°C		
	Flow rate: 1.2 mL/min		
	Mobile phase A: 0.1% TFA in water		
	Mobile phase B: ACN		
	Diluent: ACN/water (1/1, v/v)		
	Injection volume: 5µL		
	Gradient:		
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	
0.00	95	5	
9.00	5	95	
13.00	5	95	
13.10	95	5	
17.00	95	5	

Ion Chromatography (IC)

[0258] Ion Chromatography (IC) was carried out using the following instrument and parameters.

Ion Chromatography (IC)	
Instrument	Metrohm 940 professional IC
Sample center	889 IC
Detector	Conductivity detector
Eluent (anion)	3.2 mmol/L Na ₂ CO ₃ + 1.0 mmol/L NaHCO ₃
Eluent (cation)	1.7 mmol/L HNO ₃ + 0.7 mmol/L pyridinecarboxylic acid (Examples 1, 2, 3, and 6) or 2.5 mmol/L MSA (Example 4)
Suppressor solutions	2% H ₃ PO ₄
Column	Anion A SUPP 5-150 or Cation Column C4-150
Column temperature	30 °C
Flow rate	0.7 mL/min (anion) or 0.9 mL/min (cation)
Diluent	ACN/water (1/1, v/v)
Injection volume:	20 µL

Karl Fischer (KF) analysis

[0259] The water content of samples were measured via Karl Fisher (KF) analysis using the following instrument and parameters.

Karl Fischer (KF)	
Instrument	Metrohm 851/885 system
Method	Coulometric
Oven temperature	150 °C
Extraction time	120 s
Nitrogen flow	50 mL/min
Sample mass	~5-3 0mg

Differential Scanning Calorimetry (DSC)

[0260] The thermal profile of samples were measured via Differential Scanning Calorimetry (DSC) using the following instrument and parameters.

Differential Scanning Calorimetry (DSC)	
Instrument	TA Discovery 2500
Sample pan	Tzero pan and Tzero hermetic lid with a pin hole of 0.7 mm in diameter
Temperature range	30 to 250 °C or before decomposition
Heating rate	10 °C/min
Nitrogen flow	50 mL/min
Sample mass	About 1-2 mg
Modulated Differential Scanning Calorimetry (mDSC)	
Instrument	TA Instruments Discovery 2500
Sample pan	Tzero pan and Tzero hermetic lid with a manually punched pin hole of about 0.7 mm in diameter
Temperature range	0 to 200 °C
Modulation amplitude	±0.32 °C/min
Period of modulation	60 s
Heating rate	2 °C/min
Nitrogen flow	50 mL/min
Sample mass	~2-10 mg

Thermogravimetric Analysis (TGA)

[0261] Thermogravimetric Analysis (TGA) was carried out using the following instrument and parameters.

Thermal Gravimetric Analysis (TGA)	
Instrument	TA Instruments Discovery 5500
Sample pan	Aluminum, open
Start temperature	Ambient condition (below 35 °C)
Final temperature	300 °C or abort next segment if weight < 80% (w/w) (Weight loss of the compound is more than 20% (w/w).)
Heating rate	10 °C/min
Nitrogen flow	Balance 10 mL/min; sample chamber 25 mL/min
Sample mass	~2-10 mg

Dynamic Vapor Sorption (DVS)

[0262] The water sorption/desorption dynamics of samples were measured via Dynamic Vapor Sorption (DVS) the following instrument and parameters.

Dynamic Vapor Sorption (DVS)	
Instrument	Intrinsic (Examples 1, 2, 3, and 6) or SPSadv-1 μ (Example 4)
Total gas flow	200sccm (Examples 1, 2, 3, and 6) or 4000 mL/min (Example 4)
Oven temperature	25 °C
Solvent	Water
Method	Cycle: 40-0-95-0-40% RH Stage Step: 10% Equilibrium: 0.002 dm/dt (%/min) Minimum dm/dt stability duration: 60 min Maximum dm/dt stage time: 240 min
Sample mass	About 5-50 mg

Proton Nuclear Magnetic Resonance (¹H NMR)

[0263] Proton Nuclear Magnetic Resonance (¹H NMR) spectra were obtained using the following instrument and parameters.

Nuclear Magnetic Resonance (NMR)	
Instrument	Bruker Advance-AV 400M (for ¹ H-NMR)
Frequency	400 MHz
Probe	5 mm PABBO BB/19F-1H/D Z-GRD Z108618/0406
Number of scan	8
Temperature	297.6 K
Relaxation delay	1 second

[0264] List of abbreviations and acronyms

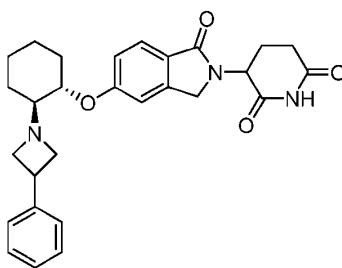
Abbreviation	Meaning
°C	degrees Celsius
ACN/MeCN	acetonitrile
AF	Amorphous Form
BPD	bis(pinacolato)diboron
EtOH	ethanol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
dr	diastereomeric ratio
EA/EtOAc	ethyl acetate
Et	ethyl

eq./equiv.	equivalent
FaSSGF	fasted state simulated gastric fluid
FaSSIF	fasted state simulated intestinal fluid
FeSSIF	fed state simulated intestinal fluid
HCl	hydrochloric acid
H ₂ O	water
HPLC	High Performance Liquid Chromatography
IPA	isopropanol
IPAc	isopropyl acetate
KF	Karl Fischer
KOAc	potassium acetate
LCMS	liquid chromatography–mass spectrometry
MEK	methyl ethyl ketone
Me	methyl
MeOH	methanol
MsOH	methanesulfonic acid
MsCl	methanesulfonyl chloride
MTBE	methyl <i>tert</i> -butyl ether
MIBK	methyl isobutyl ketone
2-MeTHF	2-methyl tetrahydrofuran
PVP K30	polyvinylpyrrolidone K30
RH	relative humidity
SEM	2-(trimethylsilyl)ethoxymethyl
SEM-Cl	2-(trimethylsilyl)ethoxymethyl chloride
t-Bu	<i>tert</i> -butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Tf	trifluoromethanesulfonyl
TLC	thin layer chromatography

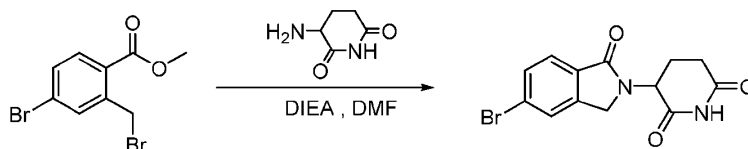
TMAP	2,2,6,6-tetramethylpiperidine
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TsCl	4-toluenesulfonyl chloride
TsOH	<i>p</i> -toluenesulfonic acid
UPCC	Ultraperformance Convergence Chromatography
V	volumes

Example 1. Polymorph Screening of Compound I

Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I free base)

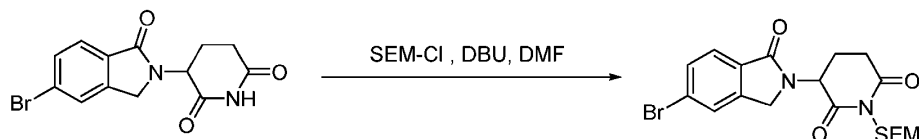


Step 1:



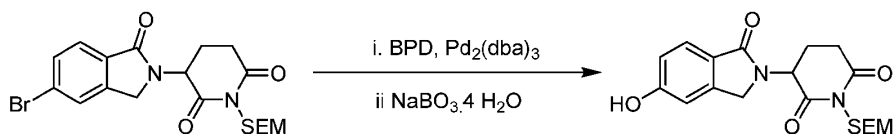
[0265] To a solution of methyl 4-bromo-2-(bromomethyl)benzoate (166.7 g, 541.29 mmol, 1 eq. in DMF (1.6 L) was added 3-aminopiperidine-2,6-dione (106.91 g, 649.55 mmol, 1.2 eq. and DIEA (279.83 g, 2.17 mol, 4 eq) in portions at 20 °C. The mixture was stirred at 120 °C for 16 hours. Another two reactions were conducted in parallel and combined for workup and purification. The reaction mixture was poured into ice-water and stirred for 20 min. The precipitated solid was filtered to give a solid. The solid was dried *in vacuo* to give 3-(5-bromo-1-oxo-isoindolin-2-yl)piperidine-2,6-dione. ¹H NMR (400 MHz, d₆-DMSO) δ 1.94 - 2.07 (m, 1 H) 2.32 - 2.45 (m, 1 H) 2.56 - 2.65 (m, 1 H) 2.89 - 2.96 (m, 1 H) 2.90 - 2.97 (m, 1 H) 3.82 (s, 1 H) 4.29 - 4.52 (m, 2 H) 5.11 (dd, *J*=13.26, 5.13 Hz, 1 H) 7.62 - 7.77 (m, 2 H) 7.89 (s, 1 H) 7.87 - 7.91 (m, 1 H) 11.00 (s, 1 H).

Step 2:



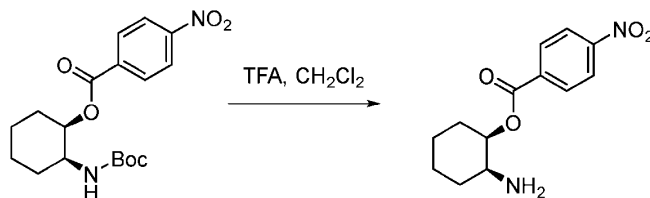
[0266] To a solution of 3-(5-bromo-1-oxo-isoindolin-2-yl)piperidine-2,6-dione (161.4 g, 499.47 mmol, 1 eq.) in DMF (2.4 L) was added DBU (152.08 g, 998.94 mmol, 2 eq.). 2-(chloromethoxy)ethyl-trimethylsilane (133.24 g, 799.15 mmol, 1.6 eq.) was added dropwise over 30 minutes at 0 °C. The mixture was stirred at 20 °C for 1 hour. Another two reactions were conducted in parallel and combined for workup and purification. The reaction mixture was poured into ice-water and stirred for 20 min. The precipitate was filtered to give a solid. The solid was dried *in vacuo* to give 3-(5-bromo-1-oxo-isoindolin-2-yl)-1-(2-trimethylsilylethoxymethyl)piperidine-2,6-dione. ¹H NMR (400 MHz, d₆-DMSO) δ -0.02 (s, 9 H) 0.75 - 0.88 (m, 2 H) 2.00 - 2.11 (m, 1 H) 2.38 (qd, *J*=13.30, 4.50 Hz, 1 H) 2.74 - 2.85 (m, 1 H) 2.98 - 3.13 (m, 1 H) 3.47 - 3.57 (m, 2 H) 4.27 - 4.35 (m, 1 H) 4.45 - 4.54 (m, 1 H) 5.05 (q, *J*=9.76 Hz, 2 H) 5.23 (dd, *J*=13.45, 5.07 Hz, 1 H) 7.65 - 7.75 (m, 2 H) 7.90 (s, 1 H).

Step 3:



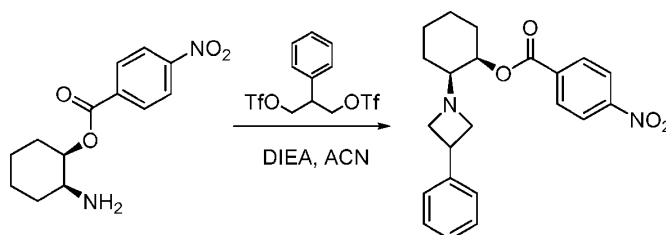
[0267] To a solution of 3-(5-bromo-1-oxo-isoindolin-2-yl)-1-(2-trimethylsilylethoxymethyl)piperidine-2,6-dione (100 g, 220.56 mmol, 1 eq.) in dioxane (500 mL) was added BPD (112.01 g, 441.11 mmol, 2 eq.) and KOAc (108.23 g, 1.10 mol, 5 eq.), Pd₂(dba)₃ (6.06 g, 6.62 mmol, 0.03 eq.) in portions at 20 °C under a nitrogen atmosphere. The mixture was stirred at 100 °C for 12 hours. The mixture was cooled to 20 °C. THF (400 mL), water (200 mL) and sodium 3-oxidodioxaborirane tetrahydrate (67.87 g, 441.11 mmol, 84.84 mL, 2 eq.) was added. The mixture was stirred at 25 °C for 4 hours. The reaction mixture was poured into water (500 mL). The mixture was extracted with ethyl acetate (3 × 500 mL). The combined organic phases were washed with brine (500 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo* to give a residue. The residue was purified by column chromatography (SiO₂, 10 to 25% ethyl acetate in petroleum ether) to give 3-(5-hydroxy-1-oxo-isoindolin-2-yl)-1-(2-trimethylsilylethoxymethyl)piperidine-2,6-dione. ¹H NMR (400 MHz, d₆-DMSO) δ -0.02 (s, 9 H) 0.77 - 0.89 (m, 2 H) 1.95 - 2.06 (m, 1 H) 2.34 (qd, *J*=13.24, 4.32 Hz, 1 H) 2.70 - 2.87 (m, 1 H) 2.95 - 3.17 (m, 1 H) 3.43 - 3.60 (m, 2 H) 4.12 - 4.21 (m, 1 H) 4.36 (d, *J*=16.88 Hz, 1 H) 5.04 (q, *J*=9.67 Hz, 2 H) 5.17 (dd, *J*=13.45, 5.07 Hz, 1 H) 6.83 - 7.00 (m, 2 H) 7.54 (d, *J*=8.25 Hz, 1 H) 10.19 (s, 1 H).

Step 4:



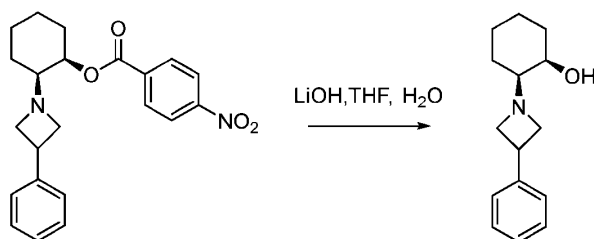
[0268] To a solution of (1R,2S)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl 4-nitrobenzoate (160 g, 439.09 mmol, 1 eq.) in DCM (1.5 L) was added TFA (462.00 g, 4.05 mol, 9.23 eq.) at 20 °C. The reaction was stirred at 20 °C for 12 hours. The mixture was concentrated in vacuo to give a residue. The crude product was triturated with ethyl acetate (300 mL). After filtration, the mother liquor was concentrated under reduced pressure to give a residue, which was triturated with 1:2 v/v ethyl acetate:methyl tertiary butyl ether (800 mL), the precipitated solid was collected by filtration, then triturated by CH₂Cl₂ (500 mL), the solid was collected by filtration and dried under reduced pressure to give [(1R,2S)-2-aminocyclohexyl] 4-nitrobenzoate. ¹H NMR (400 MHz, d₆-DMSO) δ 1.34 - 1.54 (m, 3 H) 1.61 - 1.91 (m, 4 H) 1.96 - 2.09 (m, 1 H) 3.48 (br t, *J*=6.19 Hz, 1 H) 5.25 - 5.38 (m, 1 H) 5.75 (s, 3 H) 8.29 - 8.40 (m, 4 H).

Step 5:



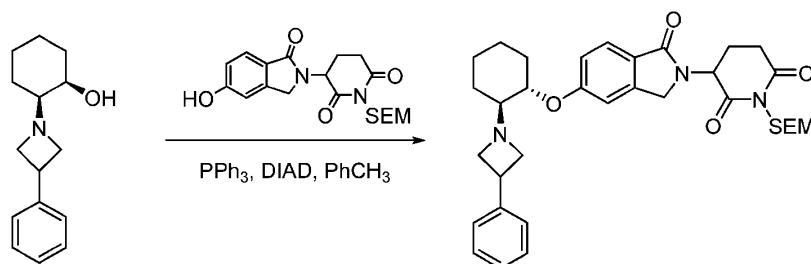
[0269] To a solution of 2-phenylpropane-1,3-diol (44 g, 289.11 mmol, 1 eq.) in MeCN (800 mL) was slowly added Tf₂O (171.30 g, 607.14 mmol, 100.17 mL, 2.10 eq.) over 20 minutes at -20 °C, followed by slow addition of DIEA (93.41 g, 722.78 mmol, 125.90 mL, 2.50 eq.) over 20 minutes. The mixture was stirred for 30 minutes. A solution of [(1R,2S)-2-aminocyclohexyl] 4-nitrobenzoate (90 g, 237.91 mmol, 1 eq., TFA salt) and DIEA (107.61 g, 832.67 mmol, 145.03 mL, 3.5 eq.) in MeCN (200 mL) was added dropwise, the mixture was stirred at 70 °C for 12 hours. The mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in EtOAc (1 L) and filtered. The filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, 10 to 50% ethyl acetate in petroleum ether) to give [(1R,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl] 4-nitrobenzoate. ¹H NMR (400 MHz, d₆-DMSO) δ 1.29-1.38 (m, 1H), 1.47-1.65 (m, 5H), 1.66-1.77 (m, 1H), 1.92-1.98 (m, 1H), 2.46 (br d, *J*=7.50 Hz, 1H), 3.01-3.19 (m, 2H), 3.43-3.71 (m, 3H), 5.20-5.30 (m, 1H), 7.10-7.34 (m, 5H), 8.15-8.42 (m, 4H).

Step 6:

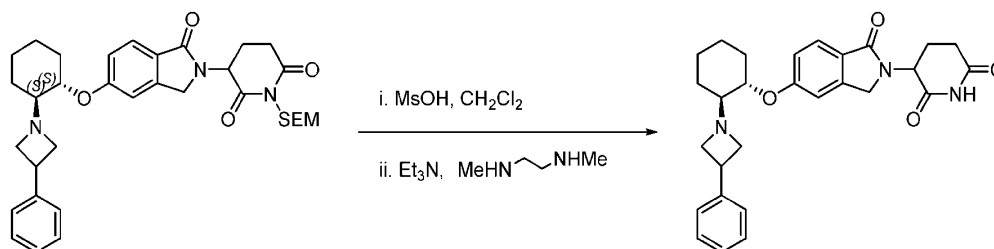


[0270] To a solution of [(1R,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl] 4-nitrobenzoate (100 g, 262.86 mmol, 1 eq.) in THF (750 mL) was added a solution of lithium hydroxide monohydrate (66.67 g, 1.59 mol, 6.04 eq.) in H₂O (750 mL). The mixture was stirred at 20 °C for 12 hours. The organic phase was separated, the aqueous layer was extracted with 1:1 v/v/ THF:MTBE (1 L). The combined organic phases were concentrated *in vacuo* to give a residue. The residue was purified by column chromatography (SiO₂, 10 to 50% ethyl acetate in petroleum ether) to give (1R,2S)-2-(3-phenylazetidin-1-yl)cyclohexanol.

Step 7:



[0271] To a solution of (1R,2S)-2-(3-phenylazetidin-1-yl)cyclohexanol (5 g, 21.61 mmol, 1 eq.), PPh₃ (9 g, 34.31 mmol, 1.59 eq.), 3-(5-hydroxy-1-oxo-isoindolin-2-yl)-1-(2-(trimethylsilyl)ethoxymethyl)piperidine-2,6-dione (5.6 g, 14.34 mmol) in anhydrous toluene (100 mL) was added DIAD (6.5 g, 32.15 mmol, 6.25 mL, 1.49 eq.) dropwise at 0 °C. After addition, the mixture was stirred at 20 °C for 12 hours. Another 7 reactions were conducted in parallel and combined for work up and purification. The mixture was filtered, the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography (SiO₂, 10 to 50% ethyl acetate in petroleum ether) to give 3-(1-oxo-5-(((1R,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione.

Step 8:

[0272] To a solution of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione. (5 g, 8.29 mmol, 1 eq.) in DCM (50 mL) was added MsOH (3.18 g, 33.1 mmol, 10.38 mL, 4 eq.) at 20 °C. The mixture was stirred for 2 hours at 20 °C. *N*¹,*N*²-dimethylethane-1,2-diamine (0.87 g, 9.9 mmol, 1.2 eq.) and TEA (8.55 g, 66 mmol, 8 eq.) was added. The mixture was stirred at 20 °C for 2 hours. The reaction mixture were filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by prep-HPLC (Column: Welch Xtimate C₁₈ 150 mm × 25 mm × 5 μm; mobile phase: [water (+ 1% TFA)-ACN]; B%: 18%-38%, 10 minute gradient) to give 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione. ¹H NMR (400 MHz, d₆-DMSO) δ 10.96 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.38 - 7.25 (m, 4H), 7.23 - 7.14 (m, 2H), 7.04 (br d, *J* = 8.4 Hz, 1H), 5.06 (dd, *J* = 4.9, 13.3 Hz, 1H), 4.45 - 4.17 (m, 3H), 3.82 - 3.46 (m, 3H), 3.30 - 3.14 (m, 2H), 2.96 - 2.82 (m, 1H), 2.59 (br d, *J* = 17.0 Hz, 1H), 2.48 - 2.27 (m, 2H), 2.09 - 1.92 (m, 2H), 1.92 - 1.80 (m, 1H), 1.66 (br s, 2H), 1.45 - 1.33 (m, 2H), 1.32 - 1.07 (m, 2H).

Characterization of Starting Material

[0273] Compound I free base, prepared by the method described above, is an approximately 1:1 diastereomeric mixture of (*R*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione and (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione. Compound I free base was analyzed by XRPD, DSC, TGA, ¹H NMR, and Karl Fisher. XRPD analysis showed that Compound I free base is crystalline (Compound I Form A).

[0274] The XRPD pattern for Compound I Form A is shown in FIG. 1. The DSC curve of Form I is shown in FIG. 2, showing an endotherm at about 207.1 °C (onset) and at about 210.0 °C (peak). The TGA of Form I is shown in FIG. 3, showing a weight loss of about 0.6 wt% up to 150 °C. The ¹H NMR of Compound I Form A indicated no detectable residual solvent. Karl Fisher analysis showed that Compound I Form A contains approximately 0.8 wt% water (0.2 molar equivalents).

[0275] Unless otherwise specified, Compound I Form A prepared by the procedure described above in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione, was used as the starting material for the polymorph screening experiments in Example 1.

Approximate solubility at 25 °C and 50 °C

[0276] About 5 mg of Compound I Form A was weighed to a 2 mL glass vial. 20 μ L aliquots of each solvent were added to dissolve the compound at 25 °C and 50 °C. Vortex and sonication were applied to assist dissolution. Maximum volume of each solvent added was 1 mL. Residual solids were determined by visual observation.

Table 1-1: Approximate solubility at 25 °C and 50 °C

Solvent	Solubility (mg/mL)	
	25 °C	50 °C
Water	<5	<5
MeOH	25-33	41-50
EtOH	5-7	14-16
IPA	<5	<5
Acetone	14-17	25-34
MEK	16-20	20-25
ACN	7-10	20-28
EA	6-8	10-12
IPAc	<5	<5
MTBE	<5	<5
DCM	>250	//
2-Me-THF	14-17	16-20
Toluene	<5	<5
Heptane	<5	<5
DMSO	16-20	71-100

//: Not carried out

Equilibration with solvents at 25 °C for 2 weeks

[0277] Based on approximate solubility results, about 40 or 50 mg of Compound I Form A was equilibrated in 0.18-0.6 mL of solvents at 25 °C for 2 weeks with a stirring bar on a magnetic stirring plate at a rate of 400 rpm.

[0278] Obtained suspensions were filtered through a 0.45 μ m nylon membrane filter by centrifugation at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. For samples with different XRPD patterns, additional analysis including HPLC, DSC, ¹H-NMR and KF was performed.

Table 1-2: Equilibration with solvents at 25°C for 2 weeks – Experiment 1

Solvent	Polymorph Form by XRPD			Additional analysis	Comments
	5 days	7 days	14 days		
Water	A	//	A	//	-
MeOH	A	//	//	//	Clear solution with longer stirring time
EtOH	A	//	A	DSC: melting onset: 210.5 °C (64 J/g)	-
Acetone	A	//	A	//	-
MEK	A	//	A	//	-
ACN	A	//	A	DSC: melting onset: 210.0 °C (80 J/g)	-
EA	A	//	A	//	-
MTBE	A	//	A	//	-
2-Me-THF	A	//	A + one peak (7.2°)	//	-
Toluene	A	//	A	//	-
DCM/IPA (1/3, v/v)	A	//	A	//	-
DMSO/IPA c (1/2, v/v)	B	//	B	¹ H-NMR: 3.5 cq. DMSO (29.6 wt%) KF: 19.5 wt% water dr: 14.9:85.1 [#]	Heated to 91 °C by TGA and cooled to room temperature under air: XRPD: Form B Heated to 150 °C by TGA and cooled to room temperature under air: XRPD: Form C
EtOH/water (1/1, v/v) a.w. = 0.89*	A	//	A	//	-
Acetone/water (1/1, v/v) a.w. = 0.85*	A	//	A	//	-

ACN/water (1/1, v/v) a.w. = 0.90*	A	//	A	//	-
DMSO/EtOH (1/2, v/v)	//	B	//	dr: 9.8:90.2 [#]	Re-slurry in DMSO/EtOH (1/2, v/v) for 7 days: XRPD: Form B dr: 2.8:97.2 [#]
DMSO/Acetone (1/2, v/v)	//	B	//	dr: 11.9:88.1 [#]	Dried under vacuum (50 °C) for 2.5 h: XRPD: Form B (low crystallinity); ¹ H-NMR: 1.4 equiv. DMSO (18.3 wt%). Above sample was dried under vacuum (50 °C) for 1.5 h: XRPD: Form B (low crystallinity); ¹ H-NMR: 1.2 equiv. DMSO (16.1 wt%). The Form B was dried under vacuum (60 °C) for 5 h: XRPD: Form B (low crystallinity) + extra peaks; ¹ H- NMR: 0.9 equiv. DMSO (14.3 wt%)
DMSO/ACN (1/2, v/v)	//	B	//	dr: 10.8:89.2 [#]	Heated to 150 °C by TGA and cooled to room temperature under air: XRPD: Form C; DSC: melting onset: 204.7 °C (88 J/g); ¹ H-NMR: 0.1 equiv. DMSO (1.9 wt%)

//: Not carried out.

*: Water activity is calculated by UNIFAC method.

#: Determined by UPCC

[0279] About 20 mg of Compound II Form B obtained from equilibration in DMSO/EtOH (1/2, v/v) at 25 °C for 2 weeks (See Table 1-2: Equilibration with solvents at 25°C for 2 weeks – Experiment 1) was equilibrated in 0.2-0.6 mL of solvents at 25 °C for 1 day with a stirring bar on a magnetic stirring plate at a rate of 400 rpm.

[0280] Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. For samples with different XRPD patterns, additional analysis including UPCC, DSC and ¹H-NMR was performed.

Table 1-3: Equilibration with solvents at 25 °C for 2 weeks – Experiment 2

Solvent	XRPD @ 1 day	Additional analysis
Water	AF	//
EtOH	Form D1	DSC: endothermic onset: 1.5 °C (16 J/g), 79.7 °C (71J/g), melting onset: 205.3 °C (21 J/g) TGA: 2.7% @ 85 °C, 5.5wt% @ (85°C -134 °C) ¹ H-NMR: 0.4 equiv. DMSO (5.5 wt%) and 1.0 equiv. EtOH (9.1 wt%) dr: 6.4:93.6 [#]
IPAc	Form B	//

//: Not carried out

#: Determined by UPCC

Equilibration with solvents at 50 °C for 1 week

[0281] Based on approximate solubility results, about 40 or 50 mg of Compound I Form A was equilibrated in 0.2-0.6 mL of solvents at 50 °C for 1 week with a stirring bar on a magnetic stirring plate at a rate of 400 rpm.

[0282] Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. For samples with different XRPD patterns, additional analysis including UPCC, DSC, TGA and ¹H-NMR was performed.

Table 1-4: Equilibration with solvents at 50 °C for 1 week

Solvent	Polymorph Form by XRPD		Additional analysis	Comments
	4 days	7 days		
Water	A	A	DSC: melting onset: 205.5 °C (67 J/g)	-
MeOH	//	//	//	Clear solution
EtOH	A	A	DSC: melting onset: 209.0 °C (37 J/g) dr: 50.8:49.3 [#]	-
Acetone	A	A	DSC: melting onset: 210.0 °C (73 J/g)	-
MEK	A	A	DSC: melting onset: 210.0 °C (74 J/g)	-
ACN	A	A	DSC: melting onset: 211.7 °C (98 J/g) dr: 50.3:49.7 [#]	-
EA	A	A	DSC: melting onset: 208.6 °C (76 J/g)	-
MTBE	A	A	DSC: melting onset: 206.0 °C (64 J/g)	-
2-Me-THF	A	A	DSC: melting onset: 206.3 °C (55 J/g)	-

Toluene	A	A	DSC: melting onset: 207.3 °C (74 J/g)	-
IPA/heptane (1/1, v/v)	A	A	DSC: melting onset: 208.7 °C (75 J/g)	-
DMSO/IPAc (1/2, v/v)	B	B	DSC: Desolvation/dehydration onset: 16.4 °C (200 J/g), 84.8 °C (15 J/g), 125.8 °C (14 J/g), melting onset: 204.7 °C (14 J/g) TGA: 8.8% @ 80 °C, 13.4 wt% @ (80 °C -124 °C), 5.4 wt% @ (124 °C -204 °C)	-
EtOH/water (1/1, v/v) a.w. = 0.88*	A	A	DSC: melting onset: 209.5 °C (72J/g)	-
Acetone/water (1/1, v/v) a.w. = 0.85*	A	A	DSC: melting onset: 207.9 °C (67J/g)	-
ACN/water (1/1, v/v) a.w. = 0.90*	A	A	DSC: melting onset: 209.1 °C (72J/g)	-
DMSO/EtOH (1/2, v/v)	//	B	dr: 10.1:89.9 [#]	Heated to 140 °C by TGA (hold for 5min) and cooled to room temperature under air: XRPD: Form C; dr: 14.9:85.1 [#] Heated to 145 °C by TGA (hold for 2min) and cooled to room temperature under air: XRPD: Form C + B
DMSO/Acetone (1/2, v/v)	//	B	dr: 14.4:85.6 [#]	-
DMSO/ACN (1/2, v/v)	//	B	dr: 11.9:88.1 [#]	-

// Not carried out.

*: Water activity is calculated by UNIFAC method.

#: Determined by UPCC

Equilibration under a temperature cycle

[0283] Based on approximate solubility results, about 40 mg of Compound I Form A was equilibrated in 0.14-0.5 mL of solvents under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min for 10 cycles. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 400 rpm.

[0284] Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD.

Table 1-5: Equilibration under a temperature cycle

Solvents	XRPD	Comments
Water	Form A	-
MeOH	//	Clear solution
EtOH	Form A	-
Acetone	Form A	-
MEK	Form A	-
ACN	Form A	-
EA	Form A	-
MTBE	Form A	-
2-Me-THF	Form A	-
Toluene	Form A	-
IPA/heptane (1/1, v/v)	Form A	-
DMSO/IPAc (1/2, v/v)	Form B	-
EtOH/water (1/1, v/v) a.w. = 0.88*	Form A	-
Acetone/water (1/1, v/v) a.w. = 0.85*	Form A	-
ACN/water (1/1, v/v) a.w. = 0.90*	Form A	-

//: Not carried out.

*: Water activity is calculated by UNIFAC method.

Crystallization at room temperature by slow evaporation

[0285] Based on approximate solubility results, about 30 mg of Compound I Form A was dissolved in 0.2-7 mL of solvent. Obtained solutions were filtered through a 0.45 µm nylon membrane filter. The clear solutions were slowly evaporated in ambient condition (about 20-25 °C, 50%-80% RH). Solid residues were investigated by XRPD.

Table 1-6: Crystallization at room temperature by slow evaporation

Solvent	XRPD	Comments
MeOH	//	Oil
EtOH	Amorphous form	-
Acetone	//	Oil

MEK	//	Oil
ACN	Amorphous form	..
EA	Form A	..
DCM	Form A	..

//: Not carried out.

Crystallization at room temperature by fast evaporation

[0286] Based on approximate solubility results, about 30 mg of Compound I Form A was dissolved in 0.4-2.6 mL of solvent. Obtained solutions were filtered through a 0.45 µm nylon membrane filter. The clear solutions were fast evaporated at room temperature (about 20-25 °C) under a dry nitrogen flow. Solid residues were investigated by XRPD.

Table 1-7: Crystallization at room temperature by fast evaporation

Solvents	XRPD	Comments
MeOH	//	Oil
EtOH	Amorphous form	-
Acetone	Form A	-
MEK	Form A	-
ACN	Amorphous form	-
EA	Form A with lower crystallinity	-
DCM	Amorphous form	-

//: Not carried out.

Crystallization from hot saturated solutions by slow cooling

[0287] Based on approximate solubility results, about 30 mg of Compound I Form A was dissolved in the minimal amount of selected solvents at 50 °C. Obtained solutions or thin suspensions were filtered through a 0.45 µm nylon membrane filter. The clear solutions were cooled to 5 °C at 0.1 °C/min. Samples without precipitates at 5 °C were further cooled to -20 °C.

[0288] Precipitates were collected by centrifugation filtration through a 0.45µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD.

Table 1-8: Crystallization from hot saturated solutions by slow cooling

Solvents	XRPD	Comments
MeOH	//	Cooled to -20 °C: clear solution
EtOH	//	Cooled to -20 °C: clear solution

Acetone	//	Cooled to -20 °C: clear solution
MEK	//	Cooled to -20 °C: clear solution
ACN	//	Cooled to -20 °C: clear solution
EA	//	Cooled to -20 °C: clear solution
2-Me-THF	Almost amorphous form	-
MeOH/toluene (2/1, v/v)	//	Cooled to -20 °C: clear solution
MeOH/MTBE (2/1, v/v)	//	Cooled to -20 °C: clear solution

//: Not carried out.

Crystallization from hot saturated solutions by fast cooling

[0289] Based on approximate solubility results, about 40 mg of Compound I Form A was dissolved in the minimal amount of selected solvents at 50 °C. Obtained solutions or thin suspensions were filtered through a 0.45µm nylon membrane filter. Obtained solutions were put into a 0 °C ice bath and agitated. Obtained clear solutions were put in the fridge at -20 °C.

Table 1-9: Crystallization from hot saturated solutions by fast cooling

Solvents	XRPD	Comments
MeOH	//	Cooled to -20 °C: clear solution
EtOH	//	Cooled to -20 °C: clear solution
Acetone	//	Cooled to -20 °C: clear solution
MEK	//	Cooled to -20 °C: clear solution
ACN	//	Cooled to -20 °C: clear solution
EA	//	Cooled to -20 °C: clear solution
2-Me-THF	//	Cooled to -20 °C: clear solution
MeOH/toluene (2/1, v/v)	//	Cooled to -20 °C: clear solution
MeOH/MTBE (2/1, v/v)	//	Cooled to -20 °C: clear solution

//: Not carried out.

Crystallization by addition of anti-solvent

[0290] Based on approximate solubility results, about 50 mg of Compound I Form A was dissolved in the minimal amount of selected good solvents at ambient temperature (about 20-25 °C). Obtained solutions or thin suspensions were filtered through a 0.45 µm syringe membrane filter. 4-10 folds of anti-solvent was added into the clear solutions slowly.

[0291] Precipitates were collected by centrifugation filtration through a 0.45 µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD.

Table 1-10: Crystallization by addition of anti-solvent

Solvent (mL)	Anti-solvent (mL)	Polymorph Form by XRPD	Comments
MeOH (1.7)	Water (6.8)	A with lower crystallinity	-
MeOH (1.7)	MTBE (17)	//	Cooled to -20 °C: clear solution
MEK (2.4)	IPAc (19.6)	//	Cooled to -20 °C: clear solution
MEK (2.4)	Heptane (9.6)	A with lower crystallinity	-
DCM (0.08)	EtOH (0.64)	A	-
DCM (0.08)	Heptane (0.64)	//	Cooled to -20 °C: clear solution
2-Me-THF (1.2)	Heptane (4.8)	A with lower crystallinity	-
DMSO (3.6)	Water (14.4)	A with lower crystallinity	-
DMSO (3.6)	IPAc (28.8)	//	Cooled to -20 °C: clear solution
DMSO (3.6)	IPA (28.8)	//	Cooled to -20 °C: clear solution

//: Not carried out.

Crystallization by reverse addition of anti-solvent

[0292] Based on approximate solubility results, about 50 mg of Compound I Form A was dissolved in the minimal amount of selected good solvents at ambient temperature (about 20-25 °C). Obtained solutions or thin suspensions were filtered through a 0.45 µm syringe membrane filter. The clear solutions were added into 4 folds of anti-solvent quickly.

[0293] Precipitates were collected by centrifugation filtration through a 0.45 µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD.

Table 1-11: Crystallization by reverse addition of anti-solvent

Solvent (mL)	Anti-solvent (mL)	Polymorph Form by XRPD	Comments
MeOH (1.7)	Water (6.8)	A with lower crystallinity	-
MeOH (1.7)	MTBE (6.8)	//	Cooled to -20 °C: clear solution
MEK (2.4)	IPAc (9.6)	//	Cooled to -20 °C: clear solution
MEK (2.4)	Heptane (9.6)	A with lower crystallinity	-
DCM (0.08)	EtOH (0.32)	//	Cooled to -20 °C: clear solution

DCM (0.08)	Heptane (0.32)	//	Cooled to -20 °C: clear solution
2-Me-THF (1.2)	Heptane (4.8)	A with lower crystallinity	-
DMSO (3.6)	Water (14.4)	A with lower crystallinity	-
DMSO (3.6)	IPAc (14.4)	//	Cooled to -20 °C: clear solution
DMSO (3.6)	IPA (14.4)	//	Cooled to -20 °C: clear solution

//: Not carried out.

Crystallization by vapor diffusion

[0294] Based on approximate solubility results, about 40 mg of Compound I Form A was dissolved in the minimal amount of selected solvents at ambient temperature (about 25 °C). Obtained solutions or thin suspensions were filtered through a 0.45 µm syringe membrane filter. The clear solutions were transferred into 4 mL glass vials without lids. Then the 4 mL vials were placed to 40 mL glass vials, respectively. To the 40 mL vials were added anti-solvent. Then the 40 mL vials were capped tightly and placed at ambient temperature for up to 30 days.

Table 1-12: Crystallization by vapor diffusion

Solvents (mL)	Anti-solvent (mL)	Comments
DMSO (3.0)	Ethanol (9.0)	Clear solution
DMSO (3.0)	EA (9.0)	Clear solution

Crystallization by heat-cool DSC

[0295] Polymorphic behavior of Compound I Form A was investigated by two different heat-cool DSC cycles.

Table 1-13: Crystallization by heat-cool DSC

Heat-cool cycles	Thermal events
Cycle 1 Step 1: 30 °C to 220 °C at 10 °C/min; Step 2: 220 °C to -20 °C at 20 °C/min; Step 3: -20 °C to 250 °C at 10 °C/min.	Step 1: melting onset: 206.4 °C (67 J/g); Step 3: glass transition T_g: 86.2 °C, ΔC_p: 0.8 J/(g.°C)
Cycle 2 Step 1: 30 °C to 220 °C at 10 °C/min; Step 2: 220 °C to -20 °C at 2 °C/min; Step 3: -20 °C to 250 °C at 10 °C/min.	Step 1: melting onset: 206.6 °C (63 J/g); Step 3: glass transition T_g: 96.9 °C, ΔC_p: 0.5 J/(g.°C)

Polymorph Screening of Compound I Results

[0296] Compound I Form A and Compound I Amorphous Form was identified in the polymorph screening. In addition, 3 polymorphs of the *S*-isomer enriched product, (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione, Compound II Form B, Compound II Form C, and Compound II Form D1, were also identified. The characterization of the Compound II polymorphs identified are described further in Example 3.

[0297] Compound I Form A is an anhydrate with a typical diastereomeric ratio of about 1:1. It is the starting material used for polymorph screening, and was obtained from most of solvent systems by equilibration, temperature cycling, slow evaporation, fast evaporation and anti-solvent addition experiments. Compound I Form A is of high crystallinity.

[0298] The XRPD pattern for Compound I Form A is shown in FIG. 1. The DSC curve of Compound I Form A is shown in FIG. 2, showing an endotherm at about 207.1 °C (onset) and about 210.0 °C (peak). The TGA of Compound I Form A is shown in FIG. 3, showing a weight loss of about 0.6 wt% up to 150 °C. The ¹H NMR of Compound I Form A indicated no detectable residual solvent. Karl Fisher analysis showed that Compound I Form A contains approximately 0.8 wt% water (0.2 molar equivalents).

[0299] Compound I Amorphous Form was obtained from ACN, EtOH and DCM by slow or fast evaporation experiments. The XRPD of Compound I Amorphous Form is shown in FIG. 4. KF analysis shows 0.5 wt% water (0.1 molar equivalent). ¹H-NMR shows 3.4 wt% (0.2 molar equivalent) of DCM. UPCC shows a diastereomeric ratio of 51.8:48.2 (*R*:*S*).

[0300] Compound I Form A is an anhydrate which showed high crystallinity and was selected for further salt screening experiments (Example 2) as well as physical and chemical stability evaluation (Example 6).

Example 2: Salt screening of Compound I

[0301] Unless otherwise specified, Compound I Form A prepared by the procedure described above in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione, was used as the starting material used for salt screening experiments in Example 2. The characterization of the starting material is described in Example 1.

Approximate solubility at 25 °C

[0302] About 5 mg of Compound I Form A was weighed to a 2 mL glass vial. 20 µL aliquots of each solvent were added to dissolve the drug substance at 25 °C. Vortex and sonication were applied to assist dissolution. Maximum volume of each solvent added was 1 mL. Approximate solubility was determined by visual observation.

Table 2-1: Approximate solubility at 25 °C

Solvents	Solubility (mg/mL)
Water	<5
MeOH	25-33
EtOH	5-7
IPA	<5
Acetone	14-17
MEK	16-20
IPAc	<5
ACN	7-10
THF	125-250
DCN	>250
MTBE	<5
DMSO	16-20

[0303] Given consideration to the solubility results, ability of the solvents to dissolve counter ions, solvent properties and previous experience, MeOH, THF, DCM, DMSO, acetone/water (49/1, v/v) and THF/water (9/1, v/v) were selected as screening solvents.

Counter ion/coformer selection

[0304] Based on the calculated pKa of 8.88 (Marvin Sketch 21.3 was used to calculate pKa), Compound I is a free base. 12 Class I acids, 2 Class II acids and 1 coformer were initially selected to pursue potential salt and cocrystal opportunities.

Table 2-2: Counter ions/coformers used for salt/cocrystal screening

Counter ions/coformers	pKa(s)*	Molecular Weight	Class
Hydrochloride acid	<-6	36.46	I
Sulfuric acid	-3	98.08	I
Phosphoric acid	1.96	98.00	I
Acetic acid	4.76	60.05	I
Succinic acid	4.21	118.09	I
Fumaric acid	3.03	116.08	I

L-Malic acid	3.46	134.09	I
Citric acid	3.13	192.13	I
Maleic acid [#]	1.92	116.08	I
Adipic acid	4.44	146.14	I
Glutaric acid	4.34	132.12	I
L-Tartaric acid	3.02	150.09	I
<i>p</i> -Toluenesulfonic acid	-1.34	172.21	II
Methanesulfonic acid	-1.2	96.10	II
Nicotinamide	N/A	122.13	Coformer

* Refer to P. Heinrich Stahl, Camille G. Wermuth, 2011. *Handbook of Pharmaceutical Salts: Properties, Selection, and Use*. 2nd ed. Wiley-VCH.

[0305] Based on screening results using the counter ions/coformers, additional 11 chiral molecules and 1 meso molecule, including 9 counter ions and 3 coformers, were selected to pursue potential salt and cocrystal opportunities and to achieve chiral resolution.

Table 2-3: Counter ions/coformers used for chiral resolution

Counter ions/coformers	pKa(s)*	Molecular Weight	Class
DL-Tartaric acid	2.96	150.09	N/A
D-Tartaric acid	2.93	150.09	N/A
L-Aspartic acid	1.88	133.11	I
<i>R</i> -(-)-Mandelic acid	3.37	152.15	III
D-(+)-Camphoric acid	4.80, 4.07 (calculated)	200.23	N/A
L-Lactic acid	3.86	90.08	I
D-Lactic acid	3.86	90.08	I
L-Ascorbic Acid	4.17	176.13	I
D-Glucuronic Acid	3.18	194.14	I
Erythritol	N/A	122.12	Coformer
D-(-)-Fructose	N/A	188.16	Coformer
D-Sorbitol	N/A	182.17	Coformer

* Refer to P. Heinrich Stahl, Camille G. Wermuth, 2011. *Handbook of Pharmaceutical Salts: Properties, Selection, and Use*. 2nd ed. Wiley-VCH.

Salt precipitation via slurry equilibration

[0306] About 50 mg of the Compound I Form A and 1.0 or 0.5 eq. of acid/coformer were added into a screening solvent in a 2 mL glass vial. Obtained mixtures were stirred at 50 °C (35 °C for DCM) for 2 hours and then at 25 °C for at least 48 hours.

[0307] Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2h, solids were analyzed by XRPD.

Table 2-4: Slurry Equilibration Experiment 1

Counter ions/coformers	MeOH	THF	DCM
Free form only	Form A	Form A	Clear solution
Hydrochloride acid, 1.0 eq.	Clear solution	Gel	Gel
Sulfuric acid, 1.0 eq.	Clear solution	AF	Clear solution
Phosphoric acid, 1.0 eq.	Clear solution	AF	Clear solution
Acetic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Succinic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Fumaric acid, 0.5 eq.	Clear solution	Fumarate salt Form A dr: 51.2:48.8 [#]	//
Fumaric acid, 1.0 eq.	Clear solution	Fumarate salt Form A	Oil
L-Malic acid, 1.0 eq.	Clear solution	Gel	Oil
Citric acid, 1.0 eq.	Clear solution	Gel	Gel
Maleic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Adipic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Glutaric acid, 1.0 eq.	Clear solution	Clear solution	Oil
L-Tartaric acid, 1.0 eq.	Clear solution	AF	L-Tartrate salt Form A dr: 84.2:15.8 [#]
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Methanesulfonic acid, 1.0 eq.	Clear solution	Gel	Clear solution
Nicotinamide, 1.0 eq.	Form A	Clear solution	Coformer
Phosphoric acid, 1.0 eq.	//	AF	//
DL-tartaric acid, 1.0 eq.	//	//	Gel

//: Not carried out.

#: Determined by UPCC

[0308] About 50 mg of Compound I Form A and 1.0 eq. of acid were added into a screening solvent in a 2 mL glass vial. Obtained mixtures were stirred at 50 °C (35 °C for DCM) for 2 hours and then at 25 °C for at least 48 hours.

[0309] Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2h, solids were analyzed by XRPD and/or UPCC.

Table 2-5: Slurry Equilibration Experiment 2

Counter ions/coformers	DMSO	THF	DCM
D-Tartaric acid, 1.0 eq.	Form B dr: 13.4:86.6 [#]	AF dr: 50.1:49.9 [#]	Gel
L-Aspartic acid, 1.0 eq.	Form B + Counter ion	Counter ion	Counter ion
<i>R</i> -(-)-Mandelic acid, 1.0 eq.	Form B dr: 13.9:86.1 [#]	<i>R</i> -mandelate salt Form A dr: 58.2:41.8 [#]	<i>R</i> -mandelate salt Form A dr: 61.2:38.8 [#]
D-(+)-Camphoric acid, 1.0 eq.	Form B	Oil	Oil
L-Lactic acid, 1.0 eq.	//	Clear solution	Clear solution
D-Lactic acid, 1.0 eq.	//	Clear solution	Clear solution

//: Not carried out.

#: Determined by UPCC

[0310] About 50 mg of Compound I Form A and 1.0 equiv. of acid/coformer were added into a screening solvent in a 2mL glass vial. Obtained mixtures were stirred at 50 °C (35 °C for DCM) for 2 hours and then at 25 °C for at least 7 days.

[0311] Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2 h, solids were analyzed by XRPD and/or UPCC.

Table 2-6: Slurry Equilibration Experiment 3

Counter ions/coformers	Acetone/water (49/1, v/v)	THF/water (9/1, v/v)	THF	DCM
L-Ascorbic acid, 1.0 eq.	//	//	Gel	Gel
D-Glucuronic acid, 1.0 eq.	//	//	Counter ion dr: 51.1:48.9 [#]	Counter ion
Erythritol, 1.0 eq.	Form C + coformer dr: 32.8:67.2 [#]	Form C + coformer	//	//
D-(-)-Fructose, 1.0 eq.	Form C + coformer	Form C dr: 31.9:68.1 [#]	//	//
D-Sorbitol, 1.0 eq.	Form C + coformer dr: 36.7:63.3 [#]	Form C	//	//

//: Not carried out.

#: Determined by UPCC

Salt precipitation via Cooling

[0312] Clear solutions obtained in slurry equilibration experiments (Table 2-4 and Table 2-5) were cooled to 5 °C to precipitate solids. Suspensions obtained after cooling were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2h, solids were analyzed by XRPD.

Table 2-7: Cooling Experiment

Counter ions/coformers	MeOH	THF	DCM
Free form only	//	//	Clear solution
Hydrochloride acid, 1.0 eq.	Clear solution	//	//
Sulfuric acid, 1.0 eq.	Clear solution	//	Clear solution
Phosphoric acid, 1.0 eq.	Clear solution	//	Clear solution
Acetic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Succinic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Fumaric acid, 0.5 eq.	Clear solution	//	//
Fumaric acid, 1.0 eq.	Clear solution	//	//
L-Malic acid, 1.0 eq.	Clear solution	//	//
Citric acid, 1.0 eq.	Clear solution	//	//
Maleic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Adipic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Glutaric acid, 1.0 eq.	Clear solution	Clear solution	//
L-Tartaric acid, 1.0 eq.	Clear solution	//	//
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Clear solution	Clear solution	Clear solution
Methanesulfonic acid, 1.0 eq.	Clear solution	//	Clear solution
Nicotinamide, 1.0 eq.	//	Potential nicotinamide cocrystal Form A	//
L-Lactic acid, 1.0 eq.	//	Clear solution	Clear solution
D-Lactic acid, 1.0 eq.	//	Clear solution	Clear solution

//: Not carried out.

Salt precipitation via anti-solvent addition

[0313] Clear solutions obtained from cooling experiments (Table 2-7) were further treated by addition of MTBE or *n*-heptane. Suspensions obtained after anti-solvent addition were filtered through a 0.45 µm nylon

membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2h, solids were analyzed by XRPD.

Table 2-8: Anti-solvent addition Experiment

Counter ions/coformers	Solvent (anti-solvent)			
	MeOH (MTBE)	THF (MTBE)	DCM (heptane)	DCM (MTBE)
Free form only	//	//	Gel	Form A
Hydrochloride acid, 1.0 eq.	Gel	//	//	//
Sulfuric acid, 1.0 eq.	Gel	//	Gel	Form A
Phosphoric acid, 1.0 eq.	Gel	//	Gel	Form A
Acetic acid, 1.0 eq.	Form A	Form A	Gel	Form A
Succinic acid, 1.0 eq.	Clear solution	Succinate salt Form A	Gel	Gel
Fumaric acid, 0.5 eq.	Gel	//	//	//
Fumaric acid, 1.0 eq.	Gel	//	//	//
L-Malic acid, 1.0 eq.	Gel	//	//	//
Citric acid, 1.0 eq.	Gel	//	//	//
Maleic acid, 1.0 eq.	Gel	Gel	Gel	Gel
Adipic acid, 1.0 eq.	Gel	AF	Gel	Gel
Glutaric acid, 1.0 eq.	Gel	AF	//	//
L-Tartaric acid, 1.0 eq.	Gel	//	//	//
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Gel	Gel	Gel	Gel
Methanesulfonic acid, 1.0 eq.	Gel	//	Gel	Gel
Nicotinamide, 1.0 eq.	//	Form A + coformer	//	//
L-Lactic acid, 1.0 eq.	//	Gel	//	Gel
D-Lactic acid, 1.0 eq.	//	Gel	//	Gel

//: Not carried out.

Salt precipitation via temperature cycle

[0314] Gels and oils obtained in slurry equilibration experiment (Table 2-4, Table 2-5, and Table 2-6) were equilibrated under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm. For gels that remained unchanged after 5 days, water (10% by volume) was added.

[0315] Suspensions obtained after cooling were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2h, solids were analyzed by XRPD.

Table 2-9: Temperature cycle Experiment 1

Counter ions/coformers	MeOH	THF	DCM
Free form only	//	//	//
Hydrochloride acid, 1.0 eq.	//	Gel	Gel
Fumaric acid, 1.0 eq.	//	Fumarate salt Form A	Gel
L-Malic acid, 1.0 eq.	//	Gel	Gel
Citric acid, 1.0 eq.	//	Gel	Gel
Glutaric acid, 1.0 eq.	//	//	Gel
Methanesulfonic acid, 1.0 eq.	//	Gel	//
Fumaric acid, 0.5 eq.	//	Fumarate salt Form A	//
Sulfuric acid, 1.0 eq.	//	Gel	//
Phosphoric acid, 1.0 eq.	//	AF	//
L-Tartaric acid, 1.0 eq.	//	Gel	//
DL-Tartaric acid, 1.0 eq.	//	//	Gel
D-Tartaric acid, 1.0 eq.	//	//	Gel
D-Camphoric acid, 1.0 eq.	//	Oil	Oil
L-Ascorbic acid, 1.0 eq.	//	Gel	Gel

//: Not carried out.

[0316] Gels obtained in anti-solvent experiments (Table 2-8) were equilibrated under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm. For gels that remained unchanged after 5 days, water (10% by volume) was added.

[0317] Suspensions obtained after cooling were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2h, solids were analyzed by XRPD and/or UPCC.

Table 2-10: Temperature cycle Experiment 2

Counter ions/coformers	Solvent (anti-solvent)			
	MeOH (MTBE)	THF (MTBE)	DCM (heptane)	DCM (MTBE)
Free form only	//	//	Form A	//
Hydrochloride acid, 1.0 eq.	Gel	//	//	//
Sulfuric acid, 1.0 eq.	Gel	//	Form A	//
Phosphoric acid, 1.0 eq.	Gel	//	Form A	//
Acetic acid, 1.0 eq.	//	//	Form A	//
Succinic acid, 1.0 eq.	//	//	Form A	

Fumaric acid, 0.5 eq.	Gel	//	//	//
Fumaric acid, 1.0 eq.	Gel	//	//	//
L-Malic acid, 1.0 eq.	Gel	//	//	//
Citric acid, 1.0 eq.	Gel	//	//	//
Maleic acid, 1.0 eq.	Gel	AF	AF	
Adipic acid, 1.0 eq.	Gel	//	AF	
Glutaric acid, 1.0 eq.	Gel	//	//	//
L-Tartaric acid, 1.0 eq.	Gel	//	//	//
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Gel	AF	AF	Gel
Methanesulfonic acid, 1.0 eq.	Gel	//	AF	AF
L-Lactic acid, 1.0 eq.	//	Form C dr:22.7:77.3 [#]	//	Gel
D-Lactic acid, 1.0 eq.	//	Gel	//	Gel

//: Not carried out.

[#]: Determined by UPCC

Salt precipitation by slow evaporation

[0318] Clear solutions obtained from cooling experiments (Table 2-7) were further treated by slow evaporation under ambient conditions. For citric acid, the solution was further treated by addition of 1,4-dioxane in an attempt to yield a solvate.

Table 2-11: Slow evaporation Experiment

Counter ions/coformers	MeOH	THF
Hydrochloride acid, 1.0 eq.	Gel	//
Sulfuric acid, 1.0 eq.	Gel	//
Phosphoric acid, 1.0 eq.	Gel	//
Succinic acid, 1.0 eq.	Gel	//
Fumaric acid, 0.5 eq.	Gel	//
Fumaric acid, 1.0 eq.	Gel	//
L-Malic acid, 1.0 eq.	Gel	//
Citric acid, 1.0 eq.	Clear solution	//
Maleic acid, 1.0 eq.	Gel	Gel
Adipic acid, 1.0 eq.	Gel	//
Glutaric acid, 1.0 eq.	Gel	//

<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Gel	Gel
Methanesulfonic acid, 1.0 eq.	Gel	//

//: Not carried out.

Characterization of salt/co-crystal hits

[0319] In total, 5 crystalline salt/cocrystal hits of Compound I were identified, including a fumarate salt Form A, an L-tartrate salt Form A, a succinate salt Form A, a *R*-mandelate salt Form A and nicotinamide cocrystal Form A. In addition, 2 polymorphs of the *S*-isomer enriched product, (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione, Compound II Form B and Compound II Form C, were also identified. The characterization of the Compound II polymorphs identified are described further in Example 3.

[0320] Compound I fumarate salt Form A was prepared by slurry and temperature cycle experiments in THF in the presence of 0.5-1.0 equivalents of fumaric acid. The XRPD of Compound I fumarate salt Form A is shown in FIG. 5. The DSC curve of Compound I fumarate salt Form A is shown in FIG. 6, showing an endotherm at about 16.1 °C (onset) and about 50.1 °C (peak), which is attributed to desolvation, and an endotherm at about 120.0 °C (onset) and about 130.9 °C (peak). The TGA of Compound I fumarate salt Form A is shown in FIG. 7, showing a weight loss of about 3.5 wt% up to 120 °C, and a further weight loss of about 8.3 wt% from 120 °C up to 173 °C.

[0321] Compound I fumarate salt Form A is a THF solvate containing about 15.5 wt% (1.43 molar equivalents) residual THF and a stoichiometric ratio of Compound I:counterion of about 1:0.77, as determined by ¹H NMR. The diastereomeric ratio of Compound I fumarate salt Form A was determined to be about 51.2:48.8 (*R*:*S*) by ultraperformance convergence chromatography (UPCC).

[0322] Compound I L-tartrate salt Form A was prepared by slurry experiments in DCM in the presence of 1.0 equivalent of L-tartaric acid. The XRPD of Compound I L-tartrate salt Form A is shown in FIG. 8. The DSC curve of Compound I L-tartrate salt Form A is shown in FIG. 9, showing an endotherm at about 16.1 °C (onset) and about 57.5 °C (peak), which is attributed to dehydration, and an endotherm at about 138.2 °C (onset) and about 155.9 °C (peak), which is attributed to melting. The TGA of Compound I L-tartrate salt Form A is shown in FIG. 10, showing a weight loss of about 5.1 wt% up to 90 °C.

[0323] Compound I L-tartrate salt Form A is a hydrate containing about 6.6 wt% water (2.4 molar equivalents) as determined by Karl Fischer analysis. Compound I L-tartrate salt Form A has a stoichiometric ratio of Compound I:counterion of about 1:0.96 and no detectable residual solvent as determined by ¹H NMR. The diastereomeric ratio of Compound I L-tartrate salt Form A was determined to be about 84.2:15.8 (*R*:*S*) by ultraperformance convergence chromatography (UPCC).

[0324] Compound I nicotinamide co-crystal Form A was prepared by slurry experiments in THF in the presence of 1.0 equivalent of nicotinamide, followed by cooling to 5 °C to precipitate solids. The XRPD of Compound I nicotinamide co-crystal Form A is shown in FIG. 11. Compound I nicotinamide co-crystal

Form A has a stoichiometric ratio of Compound I:co-former of about 1:2.23 and about 5.3 wt% (0.58 molar equivalent) residual THF as determined by ^1H NMR.

[0325] Compound I succinate salt Form A was prepared by anti-solvent addition of MTBE to THF in the presence of 1.0 equivalent of succinic acid. The XRPD of Compound I succinate salt Form A is shown in FIG. 12. The DSC curve of Compound I succinate salt Form A is shown in FIG. 13, showing an endotherm at about 14.3 °C (onset) and about 49.4 °C (peak), which is attributed to dehydration, and an endotherm at about 90.9 °C (onset) and about 113.6 °C (peak). The TGA of Compound I succinate salt Form A is shown in FIG. 14, showing a weight loss of about 3.4 wt% up to 82 °C, and a further weight loss of about 4.0 wt% from 82 °C up to 160 °C.

[0326] Compound I succinate salt Form A is a hydrate containing about 8.7 wt% water (3.1 molar equivalents) as determined by Karl Fischer analysis. Compound I succinate salt Form A has a stoichiometric ratio of Compound I:counterion of about 1:0.97 and no residual detectable solvent as determined by ^1H NMR.

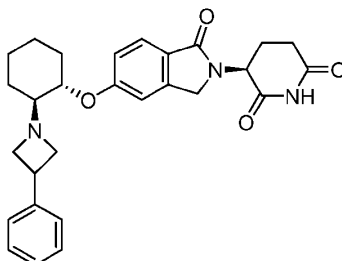
[0327] Compound I *R*-mandelate salt Form A was prepared by slurry experiments in THF in the presence of 1.0 equivalent of *R*-(-)-mandelic acid. Compound I *R*-mandelate salt Form A may also be prepared in DCM using the same method. The XRPD of Compound I *R*-mandelate salt Form A is shown in FIG. 15. The DSC curve of Compound I *R*-mandelate salt Form A is shown in FIG. 16, showing an endotherm at about 101.3 °C (onset) and about 123.1 °C (peak). The TGA of Compound I *R*-mandelate salt Form A is shown in FIG. 17, showing a weight loss of about 5.0 wt% up to 141 °C.

[0328] Compound I *R*-mandelate salt Form A is a hydrate containing about 1.8 wt% water (0.6 molar equivalents) as determined by Karl Fischer analysis. Compound I *R*-mandelate salt Form A has a stoichiometric ratio of about 1:1.02 and about 1.5 wt% (0.13 molar equivalents) residual THF as determined by ^1H NMR. The diastereomeric ratio of Compound I *R*-mandelate salt Form A was determined to be about 58.2:41.8 (*R*:*S*) by ultraperformance convergence chromatography (UPCC).

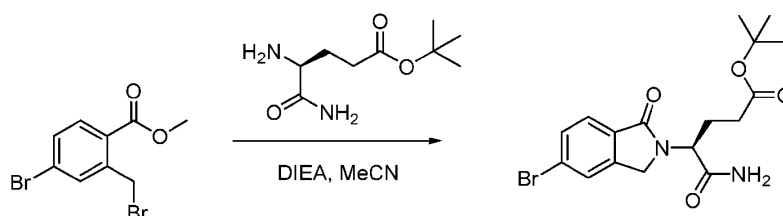
[0329] Out of the identified salts, Compound I *L*-tartrate salt Form A shows good crystallinity, high melting point, reasonable stoichiometry and counter ion safety.

Example 3: Polymorph Screening of Compound II

Synthesis of (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II)

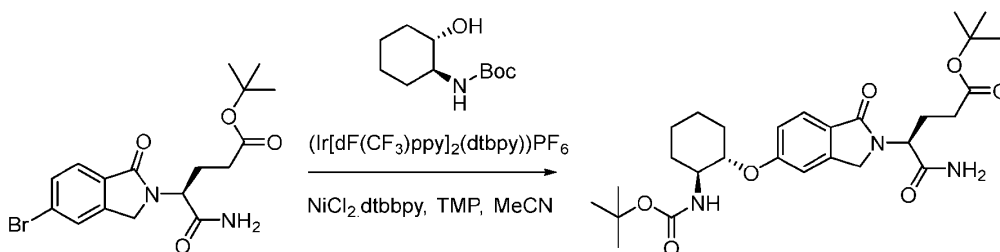


Step 1:



[0330] To a solution of methyl 4-bromo-2-(bromomethyl)benzoate (4.00 g, 12.99 mmol, 1 eq.) and *tert*-butyl (S)-4,5-diamino-5-oxopentanoate (3.10 g, 12.99 mmol, 1 eq., HCl) in MeCN (40 mL) was added DIEA (6.71 g, 51.95 mmol, 9.05 mL, 4 eq.). The mixture was stirred at 80 °C for 16 h. The reaction mixture was diluted with water (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (3 × 30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was triturated with MTBE (20 mL). The precipitate was collected by filtration and dried *in vacuo* to give *tert*-butyl (S)-5-amino-4-(5-bromo-1-oxoisindolin-2-yl)-5-oxopentanoate. ¹H NMR (400 MHz, d₆-DMSO) δ 1.33 (s, 9H), 1.90-2.04 (m, 1H), 2.07-2.27 (m, 3H), 4.44-4.51 (m, 1H), 4.57-4.65 (m, 1H), 4.70-4.76 (m, 1H), 6.93-7.41 (m, 1H), 7.61 (br d, *J*=12.38 Hz, 1H), 7.65 (s, 1H), 7.66-7.71 (m, 1H), 7.88 (s, 1H).

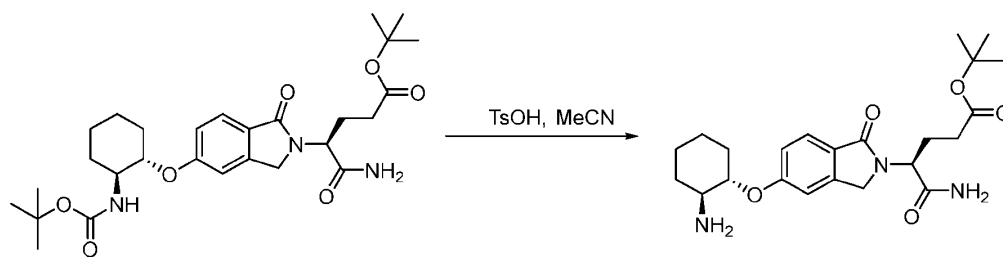
Step 2:



[0331] A solution of *tert*-butyl (S)-5-amino-4-(5-bromo-1-oxoisindolin-2-yl)-5-oxopentanoate (2.00 g, 5.03 mmol, 1 eq.), *tert*-butyl ((1S,2S)-2-hydroxycyclohexyl)carbamate (2.17 g, 10.07 mmol, 2 eq.),

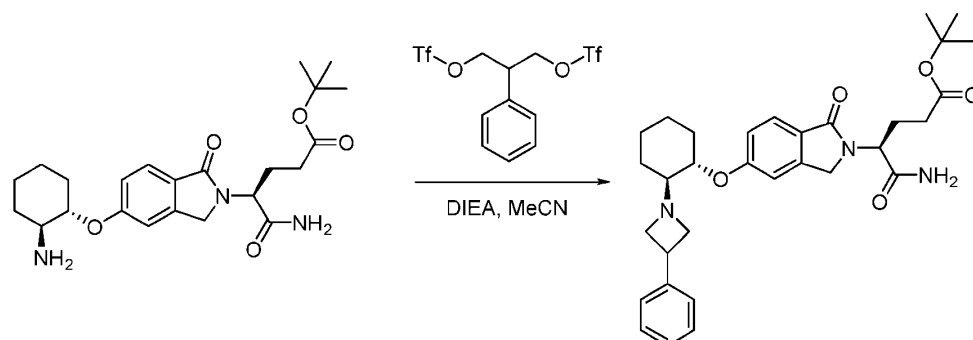
quinuclidine (55.98 mg, 503.45 μ mol, 0.1 eq.), (Ir[dF(CF₃)ppy]₂(dtbpy))PF₆ (56.48 mg, 50.34 μ mol, 0.01 eq.), NiCl₂dtbpy (100.18 mg, 251.72 μ mol, 0.05 eq.) and TMP (1.42 g, 10.07 mmol, 1.71 mL, 2 eq.) in MeCN (10 mL) was degassed three times with nitrogen. The reaction vial was sealed with parafilm, placed 2 cm away from one blue LED, and irradiated at 25 °C for 14 hours. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, 10 to 20% ethyl acetate in petroleum ether). The residue was purified by prep-HPLC (Column: Agela DuraShell C₁₈ 250 mm $\times$ 50 mm $\times$ 10 μ m; mobile phase: [water (+ 1% formic acid)-ACN]; B%: 25%-55%, 25 minute gradient) to give *tert*-butyl (*S*)-5-amino-4-(5-(((1*S*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)oxy)-1-oxoisindolin-2-yl)-5-oxopentanoate. ¹H NMR (400 MHz, d₆-DMSO) δ 1.29 (br s, 9 H) 1.33 (s, 9 H) 1.66 (br s, 2 H) 1.80 (br d, *J*=9.88 Hz, 1 H) 1.90 - 2.02 (m, 1 H) 2.04 - 2.20 (m, 4 H) 3.49 (br d, *J*=8.51 Hz, 1 H) 4.20 (br t, *J*=8.88 Hz, 1 H) 4.32 - 4.40 (m, 1 H) 4.49 - 4.58 (m, 1 H) 4.69 (br dd, *J*=10.38, 3.75 Hz, 1 H) 6.80 (br d, *J*=8.75 Hz, 1 H) 6.99 (br d, *J*=8.25 Hz, 1 H) 7.15 (br s, 2 H) 7.47 - 7.60 (m, 2 H).

Step 3:



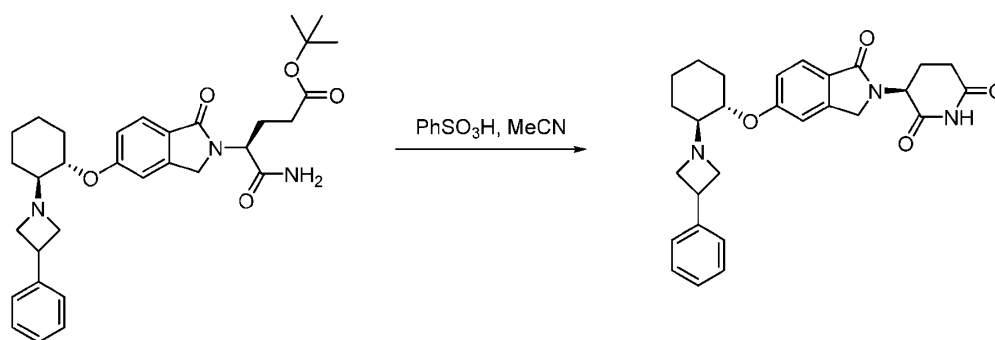
[0332] To a solution of *tert*-butyl (*S*)-5-amino-4-(5-(((1*S*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)oxy)-1-oxoisindolin-2-yl)-5-oxopentanoate (400.00 mg, 752.39 μ mol, 1 eq.) in MeCN (4 mL), was added TsOH (129.56 mg, 752.39 μ mol, 1 eq.) at 0 °C. The mixture was stirred at 20 °C for 16 hours. Additional TsOH (64.78 mg, 376.19 μ mol, 0.5 eq.) was added to the mixture. The mixture was stirred at 20 °C for 3 hours. The reaction mixture was concentrated under reduced pressure to give a residue. Water (150 mL) was added to the residue and the aqueous layer was extracted with EtOAc (3 $\times$ 50 mL). Saturated aqueous sodium bicarbonate solution was added to aqueous phase until the pH = 9. The aqueous layer was extracted with EtOAc (3 $\times$ 50 mL). The combined organic phases were washed with brine (3 $\times$ 30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give *tert*-butyl (*S*)-5-amino-4-(5-(((1*S*,2*S*)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-5-oxopentanoate which was used in the following step without further purification. ¹H NMR (400 MHz, d₆-DMSO) δ 1.21-1.29 (m, 4H), 1.31-1.35 (m, 9H), 1.59-1.72 (m, 2H), 1.85 (br d, *J*=11.38 Hz, 1H), 1.91-1.98 (m, 1H), 2.03-2.19 (m, 4H), 2.73-2.82 (m, 1H), 3.96-4.03 (m, 1H), 4.34-4.59 (m, 2H), 4.65-4.74 (m, 1H), 7.05 (br d, *J*=8.50 Hz, 1H), 7.12-7.24 (m, 2H), 7.49-7.64 (m, 2H).

Step 4:



[0333] To a solution of *tert*-butyl (*S*)-5-amino-4-(5-(((1*S*,2*S*)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-5-oxopentanoate (231.54 mg, 556.17 μ mol, 1 eq.) in MeCN (3 mL) was added DIEA (251.62 mg, 1.95 mmol, 339.11 μ L, 3.50 eq.) and 2-phenylpropane-1,3-diyl bis(trifluoromethanesulfonate) (240.00 mg, 556.17 μ mol, 1 eq.) at -20 °C. The mixture was stirred at 20 °C for 20 min. The mixture was purified directly by prep-HPLC (Column: Phenomenex C₁₈ 150 mm $\times$ 25 mm $\times$ 10 μ m; mobile phase: [water (+ NH₄HCO₃)-ACN]; B%: 45%-75%, 8 minute gradient) and lyophilized to give *tert*-butyl (*S*)-5-amino-5-oxo-4-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isindolin-2-yl)pentanoate. ¹H NMR (400 MHz, d₆-DMSO) δ 1.06-1.18 (m, 1H), 1.25-1.34 (m, 9H), 1.38 (br t, *J*=9.07 Hz, 2H), 1.65 (br s, 2H), 1.79-1.87 (m, 1H), 1.89-2.04 (m, 2H), 2.06-2.20 (m, 3H), 2.39-2.46 (m, 2H), 3.12 (t, *J*=6.75 Hz, 1H), 3.22-3.28 (m, 1H), 3.49 (quin, *J*=7.13 Hz, 1H), 3.62 (dt, *J*=19.82, 6.91 Hz, 2H), 4.24-4.31 (m, 1H), 4.32-4.54 (m, 2H), 4.65-4.71 (m, 1H), 5.22-5.25 (m, 1H), 5.39 (d, *J*=1.50 Hz, 1H), 7.01 (dd, *J*=8.44, 2.19 Hz, 1H), 7.13-7.21 (m, 2H), 7.26-7.33 (m, 3H), 7.49-7.53 (m, 1H), 7.56 (d, *J*=8.38 Hz, 1H).

Step 5:



[0334] To a solution of *tert*-butyl (*S*)-5-amino-5-oxo-4-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isindolin-2-yl)pentanoate (40 mg, 73.03 μ mol, 1 eq.) in MeCN (0.5 mL) was added benzenesulfonic acid (34.66 mg, 219.10 μ mol, 3 eq.). The reaction vessel was purged with N₂. The mixture was stirred at 80 °C for 3 hours. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (Column: Welch Xtimate C₁₈ 150 mm $\times$ 25 mm $\times$ 5 μ m; mobile phase: [water (+ 1% TFA)-ACN]; B%: 18%-38%, 10 minute gradient) and lyophilized to give

(*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione. ¹H NMR (400 MHz, d₆-DMSO) δ 1.11-1.53 (m, 4H), 1.70-1.86 (m, 2H), 1.94-2.04 (m, 1H), 2.12-2.27 (m, 2H), 2.35-2.43 (m, 1H), 2.56-2.65 (m, 1H), 2.84-2.98 (m, 1H), 3.64-3.82 (m, 2H), 3.95-4.08 (m, 1H), 4.21-4.48 (m, 5H), 4.49-4.59 (m, 1H), 5.09 (dd, *J*=13.32, 5.07 Hz, 1H), 7.17 (dd, *J*=8.44, 2.06 Hz, 1H), 7.27-7.36 (m, 2H), 7.41 (t, *J*=7.44 Hz, 2H), 7.44-7.50 (m, 2H), 7.67 (d, *J*=8.50 Hz, 1H), 10.97 (s, 1H).

[0335] (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II) synthesized from the method described above was found to be amorphous (Compound II Amorphous Form).

[0336] The XRPD of Compound II Amorphous Form is shown in FIG. 18. Compound II Amorphous Form contains no detectable residual solvent as determined by ¹H NMR. The diastereomeric ratio of Compound II Amorphous Form was determined to be about 3.8:96.2 by ultraperformance convergence chromatography (UPCC).

[0337] Unless otherwise specified, Compound II Amorphous Form prepared by the procedure described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione, was used as the starting material for the polymorph screening experiments in Example 3.

Approximate solubility at 25 °C and 50 °C

[0338] About 5 mg of Compound II Amorphous Form was weighed to a 2 mL glass vial. 20 µL aliquots of each solvent were added to dissolve the drug substance at 25 °C and 50 °C. Vortex and sonication were applied to assist dissolution. Maximum volume of each solvent added was 1 mL. Residual solids were determined by visual observation.

Table 3-1: Approximate solubility at 25 °C and 50 °C

Solvent	Solubility (mg/mL)		Comments	
	25 °C	50 °C	25 °C	50 °C
Water	<5	<5	-	-
MeOH	>250	>250	Solids precipitated out within 16 h: Form C	Solids precipitated out within 16 h: Form C
EtOH	167-250	250-500	Solids precipitated out within 16 h: Form D2, low crystallinity	-

			DSC: endothermic T _{onset} : 31.7 °C (19 J/g); 79.2 °C (26 J/g); 113.8°C (5 J/g); recrystallization T _{onset} : 156.4 °C (9 J/g); melting T _{onset} : 194.6 °C (25 J/g) dr: 6.2:93.8 [#]	
IPA	12-13	166-250	Hazy suspension	-
Acetone	125-166	250-500	-	Solids precipitated out within 16 h: Form C
MEK	62-83	62-50	Solids precipitated out within 16 h: Form C	Solids precipitated out within 16 h: Form C
ACN	11-13	11-12	-	-
EA	125-166	250-500	-	Solids precipitated out within 16 h: Form C
IPAc	10-11	11-12	-	-
MTBE	<5	<5	-	-
DCM	250-500	//	Solids precipitated out within 16 h: Form C	-
2-Me-THF	16-20	20-25	-	-
Toluene	125-166	166-250	-	Solids precipitated out within 16 h: Form C
Heptane	<5	<5	-	-
DMSO	7-8	9-10	-	-

//: Not carried out.

#: Determined by UPCC

Equilibration with solvents at 25 °C for 2 weeks

[0339] Based on approximate solubility results, about 30 mg of Compound II Amorphous Form was equilibrated in solvents at 25 °C for 2 weeks with a stirring bar on a magnetic stirring plate at a rate of 400 rpm. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. Additional analysis including HPLC, UPCC, DSC, TGA and ¹H-NMR was performed.

Table 3-2: Equilibration with solvents at 25 °C for 2 weeks

Solvents	XRPD	Additional analysis
Water	Form C	Purity: 93.8% dr (R:S): 7.7:92.3 [#]
IPA	Form C, low crystallinity	//
Acetone	Form C	Purity: 97.6% dr (R:S): 4.3:95.7 [#]
MEK	Form C	Purity: 97.2% dr (R:S): 4.7:95.3 [#]
ACN	Form C	//
EA	Form C	Purity: 96.6% dr (R:S): 5.8:94.2 [#]
IPAc	Form C	//
2-Me-THF	Form F	DSC: endothermic T _{onset} : 99.4 °C, melting T _{onset} : 208.7 °C (56 J/g) TGA: 0.8 wt% @ 95 °C, 14.2% @ (95 °C – 140 °C) ¹ H-NMR: 1.2 eq. 2-MeTHF (18.2 wt%) dr (R:S): 0.6:99.4 [#] Heated to 125 °C by TGA and cooled to room temperature under air: XRPD: Form C
EtOH/heptane (1/2, v/v)	Form C	Purity: 95.5% dr (R:S): 6.2:93.8 [#]
DCM/heptane (1/2, v/v)	Form C	//
Toluene/MTBE (1/1, v/v)	Form C	//
THF	Low crystallinity form	//
MeOH/water (1/2, v/v)	Form C	//
EtOH/water (1/2, v/v)	Form C	//
Acetone/water (1/1, v/v)	Form C	//
(R)-(-)-2-Amino-1-butanol	Low crystallinity form	//
(S)-(+)-2-Amino-1-butanol	//	Clear solution at 25 °C and after cooling to 5 °C
(R)-(+)-α-Methylbenzylamine	//	Clear solution at 25 °C and after cooling to 5 °C

//: Not carried out.

#: Determined by UPCC

Equilibration with solvents at 50 °C for 1 week

[0340] Based on approximate solubility results, about 40 mg of Compound II Amorphous Form was equilibrated in solvents at 50 °C for 1 week with a stirring bar on a magnetic stirring plate at a rate of 400 rpm. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. Additional analysis including HPLC, UPCC, DSC, TGA, PLM, ¹H-NMR and KF was performed.

Table 3-3: Equilibration with solvents at 50 °C for 1 week

Solvents	XRPD	Additional analysis	Comments
Water	Form C	Purity: 94.7% dr (R:S): 8.6:91.4 [#]	-
IPA	Form C	Purity: 97.3% dr (R:S): 5.7:94.3 [#]	-
Acetone	Form C	Purity: 96.4% dr (R:S): 7.2:92.8 [#]	-
MEK	Form C	//	-
ACN	Form C + extra peaks (5.9° and 6.2°)	Purity: 96.4%	-
Ethyl acetate	Form C	Purity: 96.5% dr (R:S): 6.2:93.8 [#]	-
IPAc	Form C	//	-
2-Me-THF	Form C	Purity: 97.4% dr (R:S): 7.4:92.6 [#]	-
EtOH/heptane (1/2, v/v)	Form C	Purity: 95.7% dr (R:S): 6.2:93.8 [#]	-
DMSO	Form B	dr (R:S): 1.8:98.2 [#]	Dried under vacuum (30 °C) for 5 h: XRPD: Form B ¹ H-NMR: 1.8 eq. DMSO (23.3 wt%) TGA: 19.9 wt % @ 138 °C, 3.7 wt % @ (138 °C – 196 °C) DSC: endothermic T _{onset} : 104.4 °C, 135.3 °C, 197.0 °C KF: 0.7 wt% water
Toluene/MTBE (1/1, v/v)	Form C	//	-
THF	Form E	DSC: endothermic T _{onset} : 12.6 °C (7 J/g), 93.4 °C (60 J/g), melting T _{onset} : 197.0 °C (4 J/g)	-

		TGA: 2.0wt % @ 80 °C, 7.3 wt% @ (80 °C -140 °C) ¹ H-NMR: 0.7 eq. THF (9.5 wt %) KF: 0.8 wt% water dr (R:S): 9.4:90.6 [#]	
MeOH/water (1/2, v/v)	Form C	Purity: 91.5% dr (R:S): 10.6:89.4 [#]	-
EtOH/water (1/2, v/v)	Form C	//	-
Acetone/water (1/1, v/v)	Form C	//	-
(R)-(-)-2-Amino-1-butanol	Low crystallinity form	//	Equilibration at 50 °C for 9 days
(S)-(+)-2-Amino-1-butanol	//	//	Clear solution at 50 °C and after cooling to 5 °C
(R)-(+)- α -Methylbenzyl amine	//	//	Clear solution at 50 °C and after cooling to 5 °C

//: Not carried out.

#: Determined by UPCC

Equilibration under a temperature cycle

[0341] Based on approximate solubility results, about 30 mg of Compound II Amorphous Form was equilibrated in solvents under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min for 10 cycles. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm. Obtained suspensions were filtered through a 0.45 μ m nylon membrane filter by centrifugation at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. For samples with different XRPD patterns, additional analysis including UPCC and DSC was performed.

Table 3-4: Equilibration under a temperature cycle

Solvent	XRPD	Additional analysis	Comments
Water	Form C	//	-
IPA	Form C	//	-
Acetone	Form C	//	-
MEK	Form C	//	-
ACN	Form C	//	-
EA	Form C	//	-
IPAc	Form C	//	-

2-Me-THF	Form C	//	-
EtOH/heptane (1/2, v/v)	Form C	//	-
DMSO	Form B	dr (R:S): 1.6:98.4 [#]	-
Toluene/MTBE (1/1, v/v)	Form C	//	-
THF	Form E	DSC: endothermic T _{onset} : 58.8 °C (10 J/g), 118.4 °C (11 J/g), 200.2 °C dr (R:S): 6.7:93.3 [#]	Heated to 100 °C by TGA and cooled to room temperature under air: XRPD: similar to Form E; DSC: endothermic T _{onset} : 113.9 °C (17 J/g), 200.2 °C; Heated to 135 °C by TGA and cooled to room temperature under air: XRPD: amorphous form
MeOH/water (1/2, v/v)	Form C	//	-
EtOH/water (1/2, v/v)	Form C	//	-
Acetone/water (1/1, v/v)	Form C	//	-

//: Not carried out.

#: Determined by UPCC

Crystallization at room temperature by slow evaporation

[0342] Based on approximate solubility results, about 20 mg of Compound II Amorphous Form was dissolved in 0.2-0.4 mL of solvents. Obtained solutions were filtered through a 0.45 µm syringe nylon membrane filter. Obtained clear solutions were slowly evaporated in ambient condition (about 20-25 °C). Solid residues were investigated by XRPD.

Table 3-5: Crystallization at room temperature by slow evaporation

Solvents	XRPD
MeOH	AF
MeOH/DCM (1:1, v/v)	AF
Acetone	AF
THF	AF
DCM	AF

Crystallization at room temperature by fast evaporation

[0343] Based on approximate solubility results, about 20 mg of Compound II Amorphous Form was dissolved in 0.2-0.4 mL of solvents. Obtained solutions were filtered through a 0.45 µm syringe membrane filter. Obtained clear solutions were fast evaporated at room temperature (about 20-25 °C) under a dry nitrogen flow. Solid residues were investigated by XRPD. For samples with different XRPD patterns, additional analysis including DSC was performed.

Table 3-6: Crystallization at room temperature by fast evaporation

Solvents	XRPD	Additional analysis	Comments
MeOH	AF	//	-
MeOH/DCM (1:1, v/v)	AF	//	-
Acetone	//	//	Gel
THF	Form G	DSC: endothermic peak starts from 44.9 °C, endothermic T _{onset} : 160.7 °C, exothermic T _{onset} : 182.1 °C	Storage at 25 °C/60% RH for about 6 days: almost AF
DCM	AF	//	-

//: Not carried out

Crystallization from hot saturated solutions by slow cooling

[0344] Based on approximate solubility results, about 30 mg of Compound II Amorphous Form was dissolved in the minimal amount of selected solvents at 50 °C. Obtained solutions were filtered through a 0.45 µm syringe membrane filter. Obtained clear solutions were cooled to 5 °C at 0.1 °C/min. Precipitates were collected by centrifugation filtration through a 0.45 µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. For samples with different XRPD patterns, additional analysis including DSC was performed.

Table 3-7: Crystallization from hot saturated solutions by slow cooling

Solvents	XRPD	Additional analysis	Comments
MeOH	Form D3	DSC: endothermic T _{onset} : 65.2 °C (97 J/g), 122.8 °C (23 J/g), 180.7 °C	-
THF	Low crystallinity form, similar to Form E	//	-
TFE	//	//	Clear solution at 5 °C and after cooling to -20 °C
Acetone/DMF (1/1, v/v)	Form C	//	-

DMF/water (1/1, v/v)	Form C	//	-
EA/DMF (1/1, v/v)	Form C	//	-
Acetone/NMP (1/1, v/v)	//	//	Storage at -20 °C for about 7 days: Form I DSC: endothermic T _{onset} : 94.1 °C (64 J/g), 186.2 °C (33 J/g) TGA: 15.9 wt% @ 91 °C, 18.1 wt% @ (91 °C – 179 °C), 7.3 wt% @ (179 °C – 250 °C) ¹ H-NMR: 0.04 eq. acetone (0.3 wt%), 3.2 eq. NMP (40 wt%)
NMP/water (1/1, v/v)	Form C	//	-
EA/NMP (1/1, v/v)	//	//	Storage at -20 °C for about 7 days: Form I dr (R:S): 1.0:99.0 [#]

//: Not carried out.

[#]: Determined by UPCC

Crystallization from hot saturated solutions by fast cooling

[0345] Based on approximate solubility results, about 30 mg of Compound II Amorphous Form was dissolved in the minimal amount of selected solvents at 50 °C. Obtained solutions were filtered through a 0.45 µm syringe membrane filter. Obtained clear solutions were put into a 0 °C ice bath and agitated. Precipitates were collected by centrifugation filtration through a 0.45µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. Obtained clear solutions were further cooled to -20 °C for crystallization.

Table 3-8: Crystallization from hot saturated solutions by fast cooling

Solvent	XRPD	Comments
MeOH	Form D3	Storage at 25 °C/93% RH for about 6 days: Form D3 with lower crystallinity
THF	Low crystallinity form, similar to Form E	-
TFE	//	Clear solution at 5 °C and after cooling to -20 °C
Acetone/DMF (1/1, v/v)	//	Storage at -20 °C for about 5 days: Form J; DSC: endothermic T _{onset} : 82.3 °C, 103.0 °C, 127.2 °C, 214.9 °C

DMF/water (1/1, v/v)	Form C with lower crystallinity	Clear solution at 5 °C and after cooling to -20 °C
EA/DMF (1/1, v/v)	//	Storage at -20 °C for about 5 days: Form J dr (R:S): 2.6:97.4 [#] Storage at 25 °C/93% RH for about 6 days: AF
Acetone/NMP (1/1, v/v)	//	Storage at -20 °C for about 5 days: Form I Storage at 25 °C/93% RH for about 10 days: Form C
NMP/water (1/1, v/v)	//	Clear solution at 5 °C and after cooling to -20 °C
EA/NMP (1/1, v/v)	//	Clear solution at 5 °C and after cooling to -20 °C

//: Not carried out.

[#]: Determined by UPCC**Crystallization by addition of anti-solvent**

[0346] Based on approximate solubility results, about 30 mg of Compound II Amorphous Form was dissolved in the minimal amount of selected good solvents at ambient temperature (about 25 °C). 4-6 folds of anti-solvent were added into the obtained clear solutions slowly. Precipitates were collected by centrifugation filtration through a 0.45 µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD.

Table 3-9: Crystallization by addition of anti-solvent

Solvent (mL)	Anti-solvent (mL)	XRPD	Comments
MeOH (0.15)	MTBE (0.6)	AF	After stirring for 1 day: Form C
EtOH (0.1)	Water (0.6)	AF	After stirring for 1 day: Form C, low crystallinity
EtOH (0.1)	Heptane (0.4)	Form C	After stirring for 1 day: Form C
Acetone (1.0)	Water (4.0)	//	After stirring for 1 day: Form C, low crystallinity
Acetone (1.0)	Heptane (2.0)	Form C, low crystallinity	After stirring for 1 day: Form C, low crystallinity
EA (0.6)	Heptane (1.2)	AF	After stirring for 1 day: Form C
EA (0.6)	MTBE (1.2)	AF	After stirring for 1 day: low crystallinity form, similar to Form H

DCM (1.0)	Heptane (3.0)	Form C, low crystallinity	After stirring for 1 day: Form C
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//: Not carried out.

Crystallization by reverse addition of anti-solvent

[0347] Based on approximate solubility results, about 30 mg of Compound II Amorphous Form was dissolved in the minimal amount of selected good solvents at ambient temperature (about 20-25 °C). Obtained solutions were filtered through a 0.45 µm syringe membrane filter. The clear solutions were added into 4 folds of anti-solvent quickly. Precipitates were collected by centrifugation filtration through a 0.45 µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD.

Table 3-10: Crystallization by reverse addition of anti-solvent

Solvents (mL)	Anti-solvent (mL)	XRPD	Comments
MeOH (0.4)	MTBE (1.6)	AF	After stirring for 3 days: AF
THF (0.5)	Water (2.0)	AF	After stirring for 3 days: Form C
THF (0.5)	Heptane (2.0)	Almost AF, similar to Form G	After stirring for 2 days: almost AF
Acetone (0.5)	Water (2.0)	AF	After stirring for 3 days: Form C, low crystallinity
MEK (0.5)	Heptane (2.0)	Almost AF, similar to Form G	After stirring for 2 days: Form C
TFE (0.2)	Water (0.8)	//	Gel
Acetone (0.5)	MTBE (2.0)	AF	After stirring for 3 days: Form G
DCM (0.7)	Heptane (2.8)	AF	After stirring for 3 days: Form C

//: Not carried out.

Crystallization by vapor diffusion

[0348] Based on approximate solubility results, about 30 mg of Compound II Amorphous Form was dissolved in the minimal amount of selected solvents in a 2 mL glass vial at ambient temperature (about 20-25 °C). Obtained solutions were filtered through a 0.45 µm syringe membrane filter. The clear solutions were transferred into 2 mL glass vials without lid. Then the 2 mL vials were placed in 20 mL glass vials. To the 20 mL vials were added anti-solvent. Then these 20 mL vials were capped tightly and placed at ambient temperature. Precipitates were collected by centrifugation filtration through a 0.45 µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were investigated by XRPD. For samples with different XRPD patterns, additional analysis including DSC, TGA, ¹H-NMR, PLM and UPCC was performed.

Table 3-11: Crystallization by vapor diffusion

Solvents (mL)	Anti-solvent (mL)	XRPD	Additional analysis	Comments
THF (0.5)	Acetone (2.0)	//	//	Clear solution
THF (0.5)	MTBE (2.0)	Form H	DSC: endothermic T _{onset} : 117.9 °C (57 J/g); exothermic T _{onset} : 179.2 °C TGA: 3.6 wt% @ 115 °C; 8.1 wt% @ (115 °C – 150 °C) ¹ H-NMR: 0.6 equiv. THF (7.8 wt%); 0.4 equiv. MTBE (6.4 wt%) dr (R:S): 1.7:98.3 [#] See Example 7 for single crystal analysis	Heated to 150 °C by TGA and cooled to room temperature under air: XRPD: AF
TFE (0.2)	Acetone (0.8)	//	//	Clear solution
TFE (0.2)	MTBE (0.8)	Almost AF	//	-
DMF/water (1/1, v/v) (0.4)	EtOH (1.6)	//	//	Clear solution

//: Not carried out

#: Determined by UPCC

Desolvation study

[0349] The interrelationship between different polymorphs were investigated by heating and cooling.

Table 3-12: Heating Experiments

Starting form	Treatment	Form by XRPD
B	Heated to 91 °C by TGA and cooled to room temperature under air	B
B	Heated to 150 °C by TGA and cooled to room temperature under air	C

D3	Heated to 112 °C by TGA and cooled to room temperature under air	D3 with lower crystallinity
D3	Heated to 150 °C by TGA and cooled to room temperature under air	AF
E	Heated to 100 °C by TGA and cooled to room temperature under air	E
E	Heated to 135 °C by TGA and cooled to room temperature under air	AF
F	Heated to 125 °C by TGA and cooled to room temperature under air	C
G	Heated to 140 °C by TGA and cooled to room temperature under air	AF
H	Heated to 150 °C by TGA and cooled to room temperature under air	AF

Polymorph screening results

[0350] Compound II Form B was prepared by equilibration and temperature cycling experiments in DMSO. The XRPD of Compound II Form B is shown in FIG. 19. The DSC curve of Compound II Form is shown in FIG. 20, showing an endotherm at about 104.4 °C (onset) and about 122.3 °C (peak), an endotherm at about 135.3 °C (onset) and about 136.5 °C (peak), and an endotherm at about 197.0 °C (onset) and about 197.4 °C (peak). The TGA of Compound II Form B is shown in FIG. 21, showing a weight loss of about 19.9 wt% up to 138 °C and a further weight loss of about 3.7 wt% from 138 °C up to 196 °C.

[0351] Compound II Form B is a DMSO solvate containing about 23.3 wt% (1.8 molar equivalents) of DMSO as determined by ¹H NMR, and about 0.7 wt% of water as determined by KF analysis. The diastereomeric ratio of Compound II Form B is about 1.8:98.2 (*R:S*) as determined by UPCC.

[0352] Compound II Form C was prepared by equilibration, temperature cycling, slow cooling, and anti-solvent experiments in several solvents such as acetone, DMF, EA, NMP, and 2-MeTHF. The XRPD of Compound II Form C is shown in FIG. 22. The DSC curve of Compound II Form C is shown in FIG. 23, showing an endotherm at about 205.6 °C (onset) and about 210.5 °C (peak), which is attributed to melting. The TGA of Compound II Form C is shown in FIG. 24, showing a weight loss of about 2.1 wt% up to 180 °C.

[0353] Compound II Form C is an anhydrate. No residual solvent was detected by ¹H NMR. The diastereomeric ratio of Compound II Form C is about 4.6:95.4 (*R:S*) as determined by UPCC.

[0354] Compound II Form D1 was prepared by equilibration in EtOH at 25 °C for 1 day. The XRPD of Compound II Form D1 is shown in FIG. 25. The DSC curve of Compound II Form D1 is shown in FIG. 26, showing an endotherm at about 74.1 °C (onset) and about 103.2 °C (peak), which is attributed to desolvation, and an endotherm at about 205.3 °C (onset) and about 210.4 °C (peak), which is attributed to melting. The TGA of Compound II Form C is shown in FIG. 27, showing a weight loss of about 2.7 wt% up to 85 °C, and a further weight loss of about 5.5 wt% from 85 °C up to 134 °C.

[0355] Compound II Form D1 is a potential EtOH solvate, containing about 5.5 wt% (0.4 molar equivalent) of DMSO and about 9.1 wt% (1.0 molar equivalent) of EtOH as determined by ¹H NMR, and about 0.5 wt% of water as determined by Karl Fischer analysis. The diastereomeric ratio of Compound II Form D1 is about 6.4:93.6 (*R:S*) as determined by UPCC.

[0356] Compound II Form D2 was prepared by precipitation from EtOH during solubility measurements. The XRPD of Compound II Form D2 is shown in FIG. 28. The DSC curve of Compound II Form D2 is shown in FIG. 29, showing an endotherm at about 31.7 °C (onset) and about 41.1 °C (peak), an endotherm at about 79.2 °C (onset) and about 98.5 °C (peak), an endotherm at about 113.8 °C (onset) and about 120.5 °C (peak), an exotherm at about 156.4 °C (onset) and about 170.4 °C (peak), which is attributed to recrystallization, and an endotherm at about 194.6 °C (onset) and about 202.9 °C (peak), which is attributed to melting.

[0357] Compound II Form D2 is a potential EtOH solvate. The diastereomeric ratio of Compound II Form D2 is about 6.2:93.8 (*R:S*) as determined by UPCC.

[0358] Compound II Form D3 was prepared by slow and fast cooling experiments in MeOH. The XRPD of Compound II Form D3 is shown in FIG. 30. The DSC curve of Compound II Form D3 is shown in FIG. 31, showing an endotherm at about 65.9 °C (onset) and about 89.5 °C (peak), and an endotherm at about 124.8 °C (onset) and about 130.5 °C (peak). The TGA of Compound II Form D3 is shown in FIG. 32, showing a weight loss of about 4.5 wt% up to 117 °C, and a further weight loss of about 0.7 wt% from 117 °C up to 250 °C.

[0359] Compound II Form D3 is a MeOH solvate containing about 5.1 wt% (0.8 molar equivalent) of MeOH as determined by ¹H NMR. The diastereomeric ratio of Compound II Form D3 is about 0.7:99.3 (*R:S*) as determined by UPCC.

[0360] Compound II Form E was prepared by equilibration at 50 °C and temperature cycling experiments in THF. The XRPD of Compound II Form E is shown in FIG. 33. The DSC curve of Compound II Form E is shown in FIG. 34, showing an endotherm at about 12.6 °C (onset) and about 40.8 °C (peak), an endotherm at about 93.4 °C (onset) and about 105.2 °C (peak), and an endotherm at about 197.0 °C (onset) and about 207.8 °C (peak), which is attributed to melting. The TGA of Compound II Form E is shown in FIG. 35, showing a weight loss of about 2.0 wt% up to 80 °C, and a further weight loss of about 7.3 wt% from 80 °C up to 140 °C.

[0361] Compound II Form E is a THF solvate containing about 9.5 wt% (0.7 molar equivalent) of THF as determined by ¹H NMR and about 0.8 wt% of water as determined by KF analysis. The diastereomeric ratio of Compound II Form E is about 9.4:90.6 (*R:S*) as determined by UPCC.

[0362] Compound II Form F was prepared by equilibration in 2-MeTHF. The XRPD of Compound II Form F is shown in FIG. 36. The DSC curve of Compound II Form F is shown in FIG. 37, showing an endotherm at about 99.4 °C (onset) and about 99.8 °C (peak), and an endotherm at about 208.7 °C (onset)

and about 214.4 °C (peak), which is attributed to melting. The TGA of Compound II Form F is shown in FIG. 38, showing a weight loss of about 0.8 wt% up to 95 °C, and a further weight loss of about 14.2 wt% from 95 °C up to 140 °C.

[0363] Compound II Form F is a 2-MeTHF solvate containing about 18.2 wt% (1.2 molar equivalents) of 2-MeTHF as determined by ¹H NMR. The diastereomeric ratio of Compound II Form F is about 0.6:99.4 (*R:S*) as determined by UPCC.

[0364] Compound II Form G was prepared by fast evaporation experiments in THF. The XRPD of Compound II Form G is shown in FIG. 39. The DSC curve of Compound II Form G is shown in FIG. 40, showing an endotherm at about 45.4 °C (onset), an endotherm at about 105.6 °C (onset) and about 116.4 °C (peak), and an exotherm at about 177.0 °C (onset). The TGA of Compound II Form G is shown in FIG. 41, showing a weight loss of about 6.0 wt% up to 150 °C and a further weight loss of about 4.3 wt% from 150 °C up to 250 °C.

[0365] Compound II Form G is a THF solvate containing about 8.3 wt% (0.6 molar equivalent) of THF as determined by ¹H NMR and about 0.25 wt% of water as determined by KF analysis. The diastereomeric ratio of Compound II Form G is about 4.0:96.0 (*R:S*) as determined by UPCC.

[0366] Compound II Form H was prepared by vapor diffusion experiments in THF/MTBE. The XRPD of Compound II Form H is shown in FIG. 42. The DSC curve of Compound II Form H is shown in FIG. 43, showing an endotherm at about 117.9 °C (onset) and about 122.3 °C (peak), and an exotherm at about 179.2 °C (onset) and about 219.6 °C (peak). The TGA of Compound II Form H is shown in FIG. 44, showing a weight loss of about 3.6 wt% up to 115 °C, and a further weight loss of about 8.1 wt% from 115 °C up to 150 °C.

[0367] Compound II Form H is a THF/MTBE solvate containing about 7.8 wt% (0.6 molar equivalent) of THF and 6.4 wt% (0.4 molar equivalent) of MTBE as determined by ¹H NMR. The diastereomeric ratio of Compound II Form H is about 1.7:98.3 (*R:S*) as determined by UPCC.

[0368] Compound II Form I was prepared by cooling experiments from NMP-containing solvents. The XRPD of Compound II Form I is shown in FIG. 48. The DSC curve of Compound II Form I is shown in FIG. 49, showing an endotherm at about 94.1 °C (onset) and about 97.8 °C (peak), and an endotherm at about 186.2 °C (onset) and 208.5 °C (peak). The TGA of Compound II Form I is shown in FIG. 50, showing a weight loss of about 15.9 wt% up to 91 °C, a further weight loss of about 18.1 wt% from 91 °C up to 179 °C, and a further weight loss of about 7.8 wt% from 179 °C up to 261 °C.

[0369] Compound II Form I is a NMP solvate containing about 40 wt% (3.2 molar equivalents) of NMP and 0.3 wt% (0.04 molar equivalent) of acetone as determined by ¹H NMR. The diastereomeric ratio of Compound II Form I is about 1.0:99.0 (*R:S*) as determined by UPCC.

[0370] Compound II Form J was prepared by cooling experiments in DMF-containing solvents. The XRPD of Compound II Form J is shown in FIG. 51. The DSC curve of Compound II Form J is shown in FIG. 52,

showing an endotherm at about 82.3 °C (onset) and 92.9 °C (peak), an endotherm at about 103.0 °C (onset) and about 107.7 °C (peak), an endotherm at about 127.1 °C (onset) and about 136.1 °C (peak), and an endotherm at about 214.9 °C (onset) and about 218.5 °C (peak).

[0371] Compound II Form J is a potential DMF solvate. The diastereomeric ratio of Compound II Form J is about 2.6:97.4 (*R:S*) as determined by UPCC.

[0372] Compound II Form C was the only polymorph identified as an anhydrate. Compound II Form C was selected for further evaluation due to its chemical and physical stability. Physical/chemical stability evaluation of Compound II Form C is further described in Example 6.

Example 4: Salt Screening of Compound II

[0373] Unless otherwise specified, Compound II Form B or Compound II Amorphous Form were used as starting materials for the polymorph screening in Example 4. The synthesis and characterization of Compound II Amorphous Form is described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione. Compound II Form B was prepared by the procedure described in Example 5, Preparation of Compound II Form B (1.8 g scale) from Compound I.

Approximate Solubility at 25 °C

[0374] The approximate solubility of Compound II Form B and Amorphous Form were determined in Example 3 (see Table 3-1). Based on the solubility results and previous experience, acetone, THF, DCM, IPA, MTBE, ACN/water (95/5, v/v) and 2-Me-THF were selected as screening solvents.

Counter ion Selection

[0375] Based on calculated pKa of 8.88, Compound II is a free base. 12 Class I acids and 4 Class II acids were selected to pursue potential salt opportunities.

Table 4-1: Counter ions used for salt screening

Counter ions	pKa(s)*	M.W.	Class
Hydrochloric acid	<-6	36.46	I
Phosphoric acid	1.96	98.00	I
Succinic acid	4.21	118.09	I
Fumaric acid	3.03	116.08	I
L-Malic acid	3.46	134.09	I
Adipic acid	4.44	146.14	I
Glycolic acid	3.28	76.05	I
L-Tartaric acid	3.02	150.09	I

Sulfuric acid	-3	98.08	I
Hippuric Acid	3.55	179.17	I
Citric acid	3.13	192.13	I
Acetic acid	4.76	60.05	I
Benzoic acid	4.19	122.12	II
Oxalic acid	1.27	90.04	II
<i>p</i> -Toluenesulfonic acid	-1.34	172.21	II
Methanesulfonic acid	-1.2	96.10	II

Salt Screening Experiments

[0376] With the selected counter ions and the selected solvents, slurry equilibration, cooling, anti-solvent addition and temperature cycling were applied as screening methods.

Slurry Equilibration

[0377] About 30 mg of Compound II Form B and 1.0 eq. of acid were added into a screening solvent in a 2 mL glass vial. Obtained mixtures were stirred at 50 °C for 2 hours and then at 25 °C for at least 4 days. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2 h, solids were analyzed by XRPD, IC, HPLC and/or UPCC.

Table 4-2: Slurry equilibration Experiment 1

Counter ions	Results (acetone)	Additional analysis
Free form only	Form C	dr (<i>R:S</i>): 1.4:98.6 [#] HPLC: 99.2%
Hydrochloric acid, 1.0 eq.	Almost AF	IC: 6.4% Cl ⁻ by weight (0.9 eq. by molar ratio)
Phosphoric acid, 1.0 eq.	Gel	//
Succinic acid, 1.0 eq.	Form C	dr (<i>R:S</i>): 1.3:98.7 [#]
Fumaric acid, 1.0 eq.	Hazy suspension	//
L-Malic acid, 1.0 eq.	Clear solution	//
Adipic acid, 1.0 eq.	Form C	//
Glycolic acid, 1.0 eq.	Form C	//

//: Not carried out

#: Determined by UPCC

[0378] About 30 mg of Compound II Form B and 1.0 eq. of acid were added into a screening solvent in a 2 mL glass vial. Obtained mixtures were stirred at 50 °C for 2 hours and then at 25 °C for at least 2 days. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2 h, solids were analyzed by XRPD.

Table 4-3: Slurry equilibration Experiment 2

Counter ions	Results (acetone)
Acetic acid, 1.0 eq.	Form C
Sulfuric acid, 1.0 eq.	Gel
Hippuric Acid, 1.0 eq.	Form C
Citric acid, 1.0 eq.	Gel
L-Tartaric acid, 1.0 eq.	Gel
Benzoic acid, 1.0 eq.	Form C
Oxalic acid, 1.0 eq.	Gel
Methanesulfonic acid, 1.0 eq.	Clear solution
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Clear solution

//: Not carried out

[0379] About 30 mg of Compound II Form B and 1.0 eq. of acid were added into a screening solvent in a 2mL glass vial. Obtained mixtures were stirred at 50 °C for 2 hours and then at 25 °C for at least 2 days. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2 h, solids were analyzed by XRPD.

Table 4-4: Slurry equilibration experiment 3

Counter ions	THF	DCM	IPA	MTBE	ACN/water (95/5, v/v)	2-MeTHF
Hydrochloric acid, 1.0 eq.	Almost AF	Form C	Almost AF	//	Clear solution	//
Phosphoric acid, 1.0 eq.	AF	Form C	AF	AF	Gel	//
Citric acid, 1.0 eq.	Gel	Gel	AF	AF	Gel	//
Fumaric acid, 1.0 eq.	Almost AF	Gel	Form C	AF + counter ion	Gel	//
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Clear solution	Clear solution	Gel	AF	Clear solution	//
L-Tartaric acid, 1.0 eq.	Gel	Gel	Form C	//	//	AF + one peak

//: Not carried out

Cooling experiments

[0380] Clear solutions obtained in slurry equilibration experiments 2 and 3 were cooled to 5 °C to precipitate solids.

Table 4-5: Cooling Experiment 1

Counter ions	Acetone
Fumaric acid, 1.0 eq.	Clear solution
L-Malic acid, 1.0 eq.	Clear solution
Methanesulfonic acid, 1.0 eq.	Clear solution
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Clear solution

[0381] Clear solutions obtained in slurry equilibration experiment 3 were cooled to 5 °C to precipitate solids

Table 4-6: Cooling Experiment 2

Counter ions	THF	DCM	IPA	MTBE	ACN/water (95/5, v/v)
Hydrochloric acid, 1.0 eq.	//	//	//	//	Clear solution
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Clear solution	Clear solution	//	//	Clear solution

//: Not carried out

Temperature Cycle

[0382] Gels obtained in slurry equilibration experiments 2 and 3 were equilibrated under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm. Obtained suspensions were filtered through a 0.45µm nylon membrane filter by centrifugation at 14,000 rpm. Obtained gels were separated from the corresponding mother liquors. After being dried at 50 °C under vacuum for 2 h, solids or gels were analyzed by XRPD, ¹H-NMR and/or IC.

Table 4-7: Temperature cycle Experiment 1

Counter ions	Acetone	Comments
Hydrochloric acid, 1.0 eq.	Almost AF	HPLC: 99.0%
Phosphoric acid, 1.0 eq.	Gel	IC: 17.5% PO ₄ ³⁻ by weight (1.1 eq. by molar ratio) HPLC: 99.0%
Sulfuric acid, 1.0 eq.	Gel	IC: 20.0% SO ₄ ²⁻ by weight (1.2 eq. by molar ratio)

		HPLC: 92.3%
Citric acid, 1.0 eq.	Gel	¹ H-NMR: free form: counter ion=1:1.10 HPLC: 99.7%
L-Tartaric acid, 1.0 eq.	Gel	¹ H-NMR: free form: counter ion=1:1.15 HPLC: 99.4%
Oxalic acid, 1.0 eq.	Gel	IC: 2.0% C ₂ O ₄ ²⁻ by weight (0.1 eq. by molar ratio) HPLC: 99.6%

[0383] Gels obtained in anti-solvent addition experiment 1 were equilibrated under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm. Obtained suspensions were filtered through a 0.45µm nylon membrane filter by centrifugation at 14,000 rpm. Obtained gels were separated from the corresponding mother liquors. After being dried at 50 °C under vacuum for 2 h, solids or gels were analyzed by XRPD, HPLC and ¹H-NMR.

Table 4-8: Temperature cycle Experiment 2

Counter ions	Acetone	Comments
Fumaric acid, 1.0 eq.	AF	¹ H-NMR: free form: counter ion=1:0.95 HPLC: 98.4%
L-Malic acid, 1.0 eq.	Gel	¹ H-NMR: free form: counter ion=1:1.20 HPLC: 98.9%
Methanesulfonic acid, 1.0 eq.	Gel	¹ H-NMR: free form: counter ion=1:1.03 HPLC: 85.8%
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Gel	¹ H-NMR: free form: counter ion=1:1.08 HPLC: 80.9%

[0384] Gel-like and amorphous samples obtained in slurry equilibration experiment 3 were equilibrated in mother liquors under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2 h, solids were analyzed by XRPD, and solids with amorphous were re-slurried in mother liquors for 7 days and 16 days.

Table 4-9: Temperature cycle Experiment 3

Counter ions	THF	DCM	IPA	MTBE	ACN/water (95/5, v/v)	2-Me- THF
Phosphoric acid, 1.0 eq.	AF [#]	//	AF [#]	Form C [#]	Gel [*]	//
Citric acid, 1.0 eq.	Gel [*]	Gel [*]	AF [#]	Almost AF [#]	Gel [*]	//
Fumaric acid, 1.0 eq.	Fumarate salt Form B [#]	Gel [*]	//	Fumarate salt Form C [#]	Gel [*]	//
<i>p</i> - Toluenesulfonic acid, 1.0 eq.	//	//	Gel [*]	AF [#]	//	//
L-Tartaric acid, 1.0 eq.	Gel [*]	Gel [*]	//	//	//	AF [#]
Hydrochloric acid, 1.0 eq.	HCl salt Form A + NaCl [#]	//	AF [#]	//	//	//

//: Not carried out

*: gel-like starting materials

amorphous starting materials

[0385] Gels obtained in anti-solvent addition experiment 2 were equilibrated under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm.

Table 4-10: Temperature cycle Experiment 4

Counter ions	THF	DCM	IPA	MTBE	ACN/water (95/5, v/v)
Hydrochloric acid, 1.0 eq.	//	//	//	//	Gel
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	//	Potential tosylate salt Form A ¹ H-NMR: free form: counter ion=1:5.4 HPLC: 89.0%	//	//	Gel

//: Not carried out

[0386] The samples from temperature cycle experiment 3 were further equilibrated under a temperature cycle between 25 °C to 40 °C by stirring at 25 °C for 4 h, heating to 40 °C at a rate of 10 °C/hour, stirring at 40 °C for 4 hours, followed by cooling to 25 °C at a rate of 10 °C/hour. The equilibration was executed with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm.

Table 4-11: Temperature cycle Experiment 5

Counter ions	THF	DCM	IPA	MTBE	ACN/water (95/5, v/v)	2-Me-THF
Citric acid, 1.0 eq.	Gel	Gel	//	//	Gel	//

//: Not carried out

Anti-solvent addition

[0387] Clear solutions obtained from cooling experiment 1 were further treated by addition of MTBE.

Table 4-12: Anti-solvent addition Experiment 1

Counter ions	Acetone
Fumaric acid, 1.0 eq.	Gel
L-Malic acid, 1.0 eq.	Gel
Methanesulfonic acid, 1.0 eq.	Gel
<i>p</i> -Toluenesulfonic acid, 1.0 eq.	Gel

[0388] Clear solutions obtained from cooling experiment 2 were further treated by addition of MTBE. Suspensions obtained after anti-solvent addition were filtered through a 0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. After being dried at 50 °C under vacuum for 2h, solids were analyzed by XRPD.

Table 4-13: Anti-solvent addition Experiment 2

Counter ions	THF	DCM	IPA	MTBE	ACN/water (95/5, v/v)
Hydrochloric acid, 1.0 equiv.	//	//	//	//	Gel
<i>p</i> -Toluenesulfonic acid, 1.0 equiv.	AF	AF	//	//	Gel

Reproduction of potential tosylate salt

[0389] Preparation of potential tosylate salt was attempted using the procedure below.

[0390] About 40 mg of Compound II Amorphous Form and 5.0 eq. of *p*-toluenesulfonic acid were added into a 2 mL glass vial. 200 µL of DCM was added and the mixture was stirred at 50 °C to give a clear solution. After stirring at 50 °C for 20 min, the solution became a hazy suspension. The suspension was kept stirring at 25 °C for 1 day and converted into a suspension. Obtained suspension was filtered through a

0.45 µm nylon membrane filter by centrifugation at 14,000 rpm. Solids were collected after being dried at 50 °C under vacuum for 3 h. The solids were re-slurried in mother liquor under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min for 5 days to give a gel. The results suggest that the potential tosylate salt was irreproducible by this method.

[0391] Preparation of potential tosylate salt was attempted using the procedure below.

[0392] About 40 mg of Compound II Amorphous Form and 1.0 eq. of *p*-toluenesulfonic acid were added into a 2 mL glass vial. 200 µL of DCM was added and the mixture was stirred at 50 °C for 2 hours and then at 25 °C for 1 hour to give a clear solution. About 400 µL of MTBE was added into above solution, then was kept stirring at 25 °C for 0.5 h to form a gel. The gel-like sample was equilibrated under a temperature cycle between 5 °C to 50 °C at a heating/cooling rate of 0.1 °C/min for 6 days and remained a gel. The results suggest that the potential tosylate salt was irreproducible by this method.

Characterization of salt hits

[0393] Compound II fumarate salt Form B was prepared by temperature cycling experiments in THF in the presence of 1.0 eq. of fumaric acid.

[0394] The XRPD of Compound II fumarate salt Form B is shown in FIG. 53. The DSC curve of Compound II fumarate salt Form B is shown in FIG. 54, showing an endotherm at about 19.8 °C (onset), an endotherm at about 118.2 °C (onset) and about 144.1 °C (peak), an endotherm at about 181.7 °C (onset) and about 192.5 °C (peak), an exotherm at about 195.5 °C (onset) and about 197.1 °C (peak), and an endotherm at about 200.7 °C (onset) and about 214.2 °C (peak). The TGA of Compound II fumarate salt Form B is shown in FIG. 55, showing a weight loss of about 3.4 wt% up to 165 °C, and a further weight loss of about 7.8 wt% from 165 °C up to 250 °C.

[0395] Compound II fumarate salt Form B is an anhydrate, containing about 0.3 wt% (0.02 molar equivalent) of THF as determined by ¹H NMR. The diastereomeric ratio of Compound II fumarate salt Form B is about 7.1:92.7 (*R*:*S*) as determined by UPCC.

[0396] Compound II fumarate salt Form C was prepared by temperature cycling experiments in MTBE in the presence of 1.0 eq. of fumaric acid.

[0397] The XRPD of Compound II fumarate salt Form C is shown in FIG. 56. The DSC curve of Compound II fumarate salt Form C is shown in FIG. 57, showing an endotherm at about 19.7 °C (onset) and about 50.9 °C (peak), an endotherm at about 99.2 °C (onset) and about 126.6 °C (peak), and an endotherm at about 161.5 °C (onset) and about 173.1 °C (peak).

[0398] Compound II fumarate salt Form C contains about 0.16 molar equivalent MTBE as determined by ¹H NMR.

[0399] Among these salt hits and polymorphs, Compound II fumarate salt Form B shows good physicochemical characteristics including medium crystallinity, high melting point, reasonable

stoichiometry and good counter ion safety. Therefore, it was selected as the salt candidate for scale-up synthesis (Example 5) and fully evaluated in comparison with Compound II Form C in terms of stability, solubility and hygroscopicity (Example 6).

Example 5: Scale-up synthesis of polymorphs

Preparation of Compound I Amorphous Form (120 mg scale)

[0400] Compound I Amorphous Form was prepared using the procedure below.

[0401] 120 mg of Compound I Form A (prepared by the procedure described in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) was weighed into an 8 mL vial. 4 mL of DCM was added into the vial under stirring with a stirring speed of 400 rpm at 25 °C. Obtained solution was filtered through a 0.22 µm nylon membrane filter. The clear solution was fast evaporated at room temperature under a dry nitrogen flow. Solid residues were investigated by XRPD. 79 mg of amorphous form was obtained in 66% yield. The sample was used for the physical and chemical stability experiments in Example 6.

Preparation of Compound I Amorphous Form (200 mg scale)

[0402] Compound I Amorphous Form was prepared using the procedure below.

[0403] 200 mg of Compound I Form A (prepared by the procedure described in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) was weighed into an 8 mL vial. 5 mL of DCM was added into the vial under stirring with a stirring speed of 400 rpm at 25 °C. Obtained solution was filtered through a 0.22µm nylon membrane filter. The clear solution was fast evaporated at room temperature under a dry nitrogen flow. 153.4 mg of Compound I Amorphous Form was obtained (77% yield). The sample was used for the physical and chemical stability experiments in Example 6.

Preparation of Compound I Amorphous Form (1.2 g scale)

[0404] Compound I Amorphous Form was prepared using the procedure below.

[0405] 1.2 g of Compound I Form A (prepared by the procedure described in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) was dissolved in 138 mL of EtOH at room 20-30 °C. Obtained solution was filtered through a 0.22µm nylon membrane filter. The solution was concentrated at 35 °C under vacuum and then dried at 20-30 °C for 18 h. The obtained dry cake was re-dissolved in 220 mL of EtOH at 25 °C and filtered through a 0.22µm nylon membrane filter. The solution was then concentrated at 35 °C under vacuum using a rotary evaporator and dried at 30-40 °C for 18. 1.54 g of Compound I Amorphous Form was obtained. The sample was used for rat PK testing in Example 8.

Preparation of Compound II Form C (200 mg scale)

[0406] Compound II Form C was prepared at 200 mg scale using the procedure below.

[0407] 0.2 g of Compound II Amorphous Form (prepared by the procedure in Example 3, Synthesis of (S)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) was weighed into an 8 mL glass vial. 1.5 mL of acetone was added into the vial at 25 °C. After stirring for about 5 min, a suspension was obtained. After stirring at 25 °C for 1 h, an additional 1.5 mL of acetone was added into the vial at 25 °C to get a better stirring.

[0408] After stirring at 25 °C for 1 day, about 1.5 mg of Compound II Form C seed crystals (prepared by slurry equilibration experiment 1 in acetone and free form only, see Table 4-2: Slurry equilibration Experiment 1) were added into the vial. The suspension was kept stirring at 25 °C for 5 days. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 4,000 rpm. Solids were collected after being dried at 50 °C under vacuum for 2 h. 145.6 mg of Compound II Form C was obtained (73% yield). The sample was used for the physical and chemical stability experiments in Example 6.

Preparation of Compound II Form C (3 g scale)

[0409] Compound II Form C was prepared at 3.0 g scale using the procedure below.

[0410] 3.0 g of Compound II Amorphous Form was weighed into a 40 mL glass vial. 10 mL of acetone was added into the vial at 25 °C. A suspension was obtained. After stirring at 25 °C for 10 min, about 3.1 mg of Compound II Form C seed crystals (prepared by the procedure described above in Example 5, Preparation of Compound II Form C (200 mg scale)) were added into the vial along with an additional 10 mL of acetone at 25 °C to get a better stirring.

[0411] The suspension was kept stirring at 25 °C for 5 days. Obtained suspension was filtered through a 0.45 µm nylon membrane filter by centrifugation at 4,000 rpm. Solids were collected after being dried at 50 °C under vacuum for 2 h and at 25 °C under vacuum for 12 h. 1.5 g of Compound II Form C was obtained (50% yield).

Preparation of Compound II Form B from Mother Liquor

[0412] The mother liquor from Example 5, Preparation of Compound II Form C (3 g scale) was dried by nitrogen purged evaporation, then the remaining solids were dissolved in DMSO. Obtained mixture was stirred at 25 °C for 3 days. Obtained suspension was filtered through a 0.45 µm nylon membrane filter by centrifugation at 4,000 rpm. Solids were collected after being dried at 50 °C under vacuum for 2 h, and was used for the single crystal cultivation and analysis in Example 7.

Preparation of Compound II Form B (1.8 g scale) from Compound I

[0413] Compound II Form B was prepared using the procedure below.

[0414] About 1.8g of Compound I Form A (prepared by the procedure described in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) was weighed into a 20 mL vial. 3.4 mL of DMSO/EtOH (1/4, v/v) was added into the vial under stirring with a

stirring speed of 400 rpm at 25 °C to form a suspension. After 3 days, a small amount of sample was centrifuged and tested by XRPD. The result showed that the material was still Compound I Form A.

[0415] After 4 days, an additional 2 mL of DMSO, 4.6 mL of DMSO/EtOH (1/3, v/v) and 5 mL of EtOH were added into the suspension in 3 portions to make the ratio of DMSO/EtOH 1:2 (v/v). The suspension was stirred at 30 °C for 1 day. After 5 days, a small amount of sample was centrifuged and tested by XRPD and UPCC. After 6 days, solid parts (wet cakes) were collected by centrifugation filtration and 657 mg of Compound II Form B was obtained. The sample was used for the physical and chemical stability experiments in Example 6.

Preparation of Compound II Form G (100 mg scale)

[0416] Compound II Form G was reproduced using the procedure below.

[0417] About 100 mg of Compound II Amorphous Form (prepared by the procedure described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) was weighed to a 4 mL glass vial. 2.0 mL of THF was added at room temperature (about 20-25 °C). Obtained solution was filtered through a 0.45 µm nylon membrane filter to give a clear solution. The clear solution was fast evaporated at room temperature (about 20-25 °C) under a dry nitrogen flow for about 15 hours, and the solid parts were characterized.

Preparation of Compound II Form D3 (180 mg scale)

[0418] Compound II Form D3 was reproduced using the procedure below.

[0419] About 100 mg of Compound II Amorphous Form (prepared by the procedure described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) was weighed to a 4 mL glass vial. 0.9 mL of MeOH was added at 50 °C. Obtained solution was filtered through a 0.45 µm nylon membrane filter. The clear solution was cooled to 5 °C at 0.1 °C/min. After stirring for about 12 hours at 5 °C, precipitates were collected by centrifugation filtration through a 0.45 µm nylon membrane filter at 14,000 rpm. Solid parts (wet cakes) were characterized.

Preparation of Compound II fumarate salt Form B (200 mg scale)

[0420] Compound II fumarate salt Form B was prepared using the procedure below.

[0421] 0.2 g of Compound II Amorphous Form (prepared by the procedure described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) and 1.1 eq. of fumaric acid were added in an 8 mL glass vial. 1.0 mL of THF was added into the vial, obtained mixtures were stirred at 50 °C for 20 min, and a clear solution was obtained. About 2.1 mg of the Compound II fumarate salt Form B seed crystals (prepared by temperature cycle experiment 1 with 1.0 equiv. fumaric acid in THF, see Table 4-9: Temperature cycle Experiment 3) were added into above solution. After stirring at 50 °C for 20 min, the solution became cloudy and then gradually converted into a

suspension. The suspension was cooled to 25 °C, and kept under stirring at 25 °C for 3 h, and an additional 1.0 mL of THF was added into the vial to get a better stirring.

[0422] The suspension was kept stirring at 25 °C for 5 days. Obtained suspension was filtered through a 0.45 µm nylon membrane filter by centrifugation at 4,000 rpm. Solids were collected after being dried at 50 °C under vacuum for 2 h. 163.7 mg of the Compound II fumarate salt Form B was obtained as an off-white solid (65% yield). The sample was used for the physical and chemical stability experiments in Example 6.

Preparation of Compound II fumarate salt Form B (800 mg scale)

[0423] Compound II fumarate salt Form B was prepared using the procedure below.

[0424] Compound II Amorphous Form (prepared by the procedure described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione), was equilibrated in water at 25 °C for 30 min with a stirring bar on a magnetic stirring plate at a rate of 400 rpm. Obtained suspensions were filtered through a 0.45 µm nylon membrane filter by centrifugation at 4,000 rpm. Then the wet cake was washed with 1 mL water. Solids were collected after being dried at 25 °C under vacuum for 16 h.

[0425] Solids obtained (about 0.8 g), and 1.05 equiv. of fumaric acid were added in a 20 mL glass vial. 4 mL of THF was added into the vial, obtained mixtures were stirred at 50 °C for 5 min, and a clear solution was obtained. About 2.3mg of Compound II fumarate salt Form B seed crystals (prepared by temperature cycle experiment 1 with 1.0 equiv. fumaric acid in THF, see Table 4-9: Temperature cycle Experiment 3) were added into above solution. After stirring at 50 °C for 10 min, the solution became cloudy and then gradually converted into a suspension.

[0426] The suspension was cooled to 25 °C, then kept under stirring for 3 days. Obtained suspension was filtered through a 0.45 µm nylon membrane filter by centrifugation at 4,000 rpm. Then the wet cake was washed with 1.5 mL THF. Solids were collected after being dried at 25 °C under vacuum for 12h. 513 mg of Compound II fumarate salt Form B was obtained in 51.3% yield. The sample was used for rat PK testing in Example 8.

Example 6: Additional Physical/Chemical Properties of Compound I and Compound II

[0427] Unless otherwise specified, Compound I Form A (prepared by the procedure described in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione) and Compound I Amorphous Form (prepared by the procedure described in Example 5, Preparation of Compound I Amorphous Form (120 mg scale) or Preparation of Compound I Amorphous Form (200 mg scale)) were used as starting materials for the studies in Example 6.

[0428] Unless otherwise specified, Compound II Amorphous Form (prepared by the procedure described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione), Compound II Form C (prepared by the procedure described in Example 5, Preparation of Compound II Form C (200 mg scale)), and Compound II fumarate salt Form B (prepared by

the procedure described in Example 5, Preparation of Compound II fumarate salt Form B (200 mg scale)) were used as starting materials for the studies in Example 6.

Bulk Stability of Compound I

[0429] Compound I Form A and Compound I Amorphous Form were placed at 25 °C/93% RH in an open container, at 40 °C/75% RH in an open container and at 60 °C in a closed container for 1 week. Samples after the stress were characterized by XRPD and HPLC and inspected for color change.

Table 6-1: Bulk Stability of Compound I Form A and Amorphous Form

Compound I Polymorph	Form A		Amorphous Form	
Initial purity	97.9%		96.5%	
	Purity	Color	Purity	Color
Solid state, 25 °C/93% RH in an open container, 1 week				
Bulk (HPLC)	96.8%	No change	96.8%	No change
Bulk (XRPD)	Form A		Amorphous Form	
Solid state, 40 °C/75% RH, in an open container, 1 week				
Bulk (HPLC)	97.2%	No change	97.3%	No change
Bulk (XRPD)	Form A		Amorphous Form	
Solid state, 60 °C, in a tight container, 1 week				
Bulk (HPLC)	96.9%	No change	97.0%	No change
Bulk (XRPD)	Form A		Amorphous Form	

Solubility of Compound I

[0430] About 7 mg of Compound I Form A or 7 mg of Compound I Amorphous Form was weighed into an 8 mL glass vial. 3.5mL of solubility medium was added. Obtained suspensions/solutions were stirred at 37 °C at 400 rpm for 24 hours and then centrifuged at 37 °C at 14,000 rpm for 5 min. Supernatants were analyzed by HPLC and pH meter. Residual solids (wet cakes) were characterized by XRPD to determine physical form.

Table 6-2: Solubility of Compound I Form A and Amorphous Form

Compound I Form A				
Solubility media	Solubility, mg/mL		Form by XRPD	Additional analysis
	2h	24h (pH)		
pH 1.2 HCl solution (0.2 N)	>2	>2 (1.21)	//	HPLC: 96.7%

pH 4.5 acetate buffer (50 mM)	1.9	2.0 (4.47)	A	//
pH 6.8 phosphate buffer (50 mM)	0.26	0.33 (6.69)	A	//
FaSSGF, pH 1.6	>2	>2 (1.81)	//	//
FaSSIF, pH 6.5	0.67	0.70 (6.30)	A	//
FeSSIF, pH 5.0	1.9	2.0 (5.10)	//	//
Water	0.02	0.03 (7.68)	A	//
Compound I Amorphous Form				
Solubility media	Solubility, mg/mL		Form by XRPD	Additional analysis
	2h	24h (pH)		
pH 1.2 HCl solution (0.2 N)	>2	>2 (1.29)	//	HPLC: 96.6%
pH 4.5 acetate buffer (50 mM)	2.0	>2 (4.49)	//	//
pH 6.8 phosphate buffer (50 mM)	0.59	0.69 (6.70)	A	//
FaSSGF, pH 1.6	1.9	>2 (1.82)	//	//
FaSSIF, pH 6.5	1.6	1.7 (6.36)	AF	//
FeSSIF, pH 5.0	1.5	>2 (5.13)	//	//
Water	0.04	0.10 (7.79)	AF	//

//: Not carried out

Bulk Stability of Compound II Form C and Compound II fumarate salt Form B

[0431] Compound II Form C and Compound II fumarate salt Form B were placed at 25 °C/93% RH in an open container, at 40 °C/75% RH in an open container and at 60 °C in a closed container for 1 week. Samples after the stress were characterized by XRPD, HPLC and UPCC and inspected for color change.

Table 6-3: Bulk Stability of Compound II Form C and Compound II fumarate salt Form B

	Compound II Form C		Compound II fumarate salt Form B	
Initial purity by HPLC	97.5%		98.0%	
dr (R:S) by UPCC	4.6:95.4		7.1:92.9	
Initial color	Off-white		Off-white	
	Purity	Color change	Purity	Color change
Solid state, 25 °C/93% RH, open container, 1 week				
Bulk (HPLC)	97.2%	No change	97.3%	No change
dr (R:S) by UPCC	5.7:94.3		6.1:93.9	

Bulk (XRPD)	Form C		Fumarate salt Form B	
Solid state, 40 °C/75% RH, open container, 1 week				
Bulk (HPLC)	97.1%	No change	97.1%	No change
dr (R:S) by UPCC	5.2:94.8		6.6:93.4	
Bulk (XRPD)	Form C		Fumarate salt Form B	
Solid state, 60 °C, tight container, 1 week				
Bulk (HPLC)	97.2%	No change	96.7%	No change
dr (R:S) by UPCC	5.7:94.3		11.7:88.3	
Bulk (XRPD)	Form C		Fumarate salt Form B	

Solubility of Compound II Amorphous Form

[0432] About 5 mg of the Compound II Amorphous Form was weighed into an 8 mL glass vial, respectively. 2.5 mL of solubility medium was added. Obtained suspensions/solutions were stirred at 37 °C at 400 rpm and sampled at 2 hours and at 24 hours. The samples were centrifuged at 37 °C at 14,000 rpm for 5 min. Supernatants were analyzed by HPLC and pH meter. Residual solids (wet cakes) from the 24 hours samples were also characterized by XRPD to determine physical form. Solutions obtained from the HCl (pH=1.2) experiment were also analyzed by HPLC and UPCC, after storage at room temperature for 29 days.

Table 6-4: Solubility of Compound II Amorphous Form

Solubility media	Compound II Amorphous Form		
	Solubility, mg/mL		Analysis
	2h	24h (pH)	
pH 1.2 HCl solution	>2	>2 (1.33)	HPLC: 89.3% dr (R:S): 6.5:93.5 [#]
pH 4.5 acetate buffer (50mM)	1.8	1.8 (4.67)	//
pH 6.8 phosphate buffer (50mM)	0.70	0.66 (6.89)	AF
FaSSGF, pH 1.6	>2	>2 (1.72)	//
FaSSIF-v1, pH 6.5	1.0	0.34 (6.64)	Form C
FeSSIF-v1, pH 5.0	>2	>2 (5.09)	//
Water	0.018	0.068 (7.27)	AF

//: Not carried out

#: Determined by UPCC

Solubility of Compound II Form C and Compound II fumarate salt Form B

[0433] About 7 mg of Compound II Form C or 8.71 mg of fumarate salt Form B was weighed into 8 mL glass vials. The salt amount used is equivalent to 7 mg anhydrous free form. 3.5 mL of solubility medium was added. Obtained suspensions/solutions were stirred at 37 °C at 400 rpm and sampled at 2 hours and at 24 hours. The samples were centrifuged at 37 °C at 14,000 rpm for 5 min. Supernatants were analyzed by HPLC and pH meter. Residual solids (wet cakes) from the 24 hours samples were also characterized by XRPD to determine physical form. Solutions obtained from the HCl (pH=1.2) experiment were also analyzed by HPLC and UPCC, after storage at room temperature for 29 days.

Table 6-5: Solubility of Compound II polymorphs

Compound II polymorph	Form C, anhydrate			Fumarate salt Form B, anhydrate		
	Solubility, mg/mL		Analysis	Solubility, mg/mL		Analysis
	2h	24h (pH)		2h	24h (pH)	
pH 1.2 HCl solution	>2	>2 (1.21)	HPLC: 94.5% dr (R:S): 4.9:95.1 [#]	>2	>2 (1.20)	HPLC: 94.5% dr (R:S): 7.2:92.8 [#]
pH 4.5 acetate buffer (50mM)	1.8	1.8 (4.61)	//	>2	>2 (4.30)	//
pH 6.8 phosphate buffer (50mM)	0.062	0.086 (6.75)	Form C	1.6	1.6 (6.58)	//
FaSSGF, pH 1.6	>2	>2 (1.58)	//	>2	>2 (1.59)	//
FaSSIF-v1, pH 6.5	0.13	0.15 (6.47)	Form C	1.8	1.8 (6.07)	//
FeSSIF-v1, pH 5.0	>2	>2 (4.99)	//	>2	>2 (4.88)	//
Water	0.006	0.012 (7.18)	Form C	>2	>2 (3.66)	//

//: Not carried out

#: Determined by UPCC

Hygroscopicity of Compound II Form B

[0434] Water sorption and desorption behavior of Compound II Form B was investigated by dynamic vapor sorption (DVS) at 25 °C with a cycle of 40-95-0-95-40%RH. XRPD was measured after the DVS test to determine form change.

Table 6-6: Water sorption and desorption behavior of Compound II Form B

Method	40-95-0-95-40% RH, dm/dt 0.002%/min, minimum equilibration time 60 min per step, maximum equilibration time 360 min per step, 25 °C			
Relative humidity at 25 °C	1 st sorp. wt% change	1 st desorp. wt% change	2 nd sorp. wt% change	2 nd desorp. wt% change
0%	N/A	5.0	5.0	N/A
10%	N/A	5.6	5.3	N/A
20%	N/A	6.1	5.5	N/A
30%	N/A	6.5	5.7	N/A
40%	14.8	7.0	5.9	0.0
50%	14.8	7.8	6.1	0.7
60%	14.8	8.7	6.2	1.7
70%	14.8	9.8	6.4	3.3
80%	14.4	15.9	6.8	8.2
90%	15.8	35.7	8.7	23.4
95%	46.7	46.7	34.6	34.6
Hygroscopicity				
Non-hygroscopic* from 40% to 80% RH at 25 °C				
XRPD after DVS test				
Form B with low crystallinity				

*Non-hygroscopic: water uptake <0.2%

Hygroscopicity of Compound II Form C and Compound II fumarate salt Form B

[0435] Water sorption and desorption behavior of Compound II Form C and Compound II fumarate salt Form B was investigated by DVS at 25 °C. Samples were held at each step for 240 min.

Table 6-7: Hygroscopicity of Compound II Form C and Compound II fumarate salt Form B

RH 25 °C	Compound II Form C				Compound II fumarate salt Form B			
	Desorp. (%)	Sorp. (%)	Desorp. (%)	Sorp. (%)	Desorp. (%)	Sorp. (%)	Desorp. (%)	Sorp. (%)
0%	0.4	0.4	0.1	0.1	0.1	0.1	0.2	0.2
10%	0.5	0.5	0.2	0.3	0.6	0.5	0.9	0.7
20%	0.6	0.7	0.4	0.5	0.8	0.7	1.3	1.2

30%	0.7	0.7	0.5	0.4	1.1	0.8	1.5	1.3
40%	0.9	0.8	0.7	0.6	1.3	1.1	1.9	1.4
50%	//	1.0	1.6	//	//	1.3	2.2	//
60%	//	1.3	2.3	//	//	1.6	2.6	//
70%	//	2.2	2.9	//	//	2.2	4.4	//
80%	//	4.0	3.9	//	//	4.3	6.3	//
90%	//	6.2	6.4	//	//	7.5	10.8	//
95%	//	10.5	10.5	//	//	18.8	18.8	//
Hygroscopicity	Moderately hygroscopic, 3.2 wt% water uptake from 40% to 80% RH				Moderately hygroscopic, 3.2 wt% water uptake from 40% to 80% RH			
XRPD after DVS test	Form C				Fumarate salt Form B			

//: Not carried out

Compression simulation of Compound II Form C

[0436] About 10 mg of Compound II Form C was compressed for 5 minutes under 2 MPa, 5 MPa and 10 MPa with a hydraulic press. Potential form change and degree of crystallinity were evaluated by XRPD.

Table 6-8: Compression simulation of Compound II Form C

Pressure	XRPD	Comments
10 MPa	Form C	Slight decrease in crystallinity by showing broader peaks
5 MPa	Form C	Slight decrease in crystallinity by showing broader peaks
2 MPa	Form C	No obvious change in crystallinity

Dry grinding simulation of Compound II Form C

[0437] About 10 mg of Compound II Form C was ground manually with a mortar and a pestle for 1 min, 3 min and 5 min. Potential form change and degree of crystallinity were evaluated by XRPD.

Table 6-9: Dry grinding simulation of Compound II Form C

Grinding time	XRPD	Comments
5 min	Form C	Slight decrease in crystallinity by showing broader peaks

3 min	Form C	Slight decrease in crystallinity by showing broader peaks
1 min	Form C	No obvious change in crystallinity

Wet granulations simulation of Compound II Form C

[0438] Water or ethanol was added drop wise to about 10 mg of Compound II Form C until the sample is wetted sufficiently. Wet sample was ground gently with in a mortar and a pestle for 5 min. Post granulation sample was dried under ambient condition for 10 min. Potential form change and degree of crystallinity were evaluated by XRPD.

Table 6-10: Wet granulations simulation of Compound II Form C

Granulation solvents	XRPD	Comments
Water	Form C	Slight decrease in crystallinity by showing broader peaks
Ethanol	Form C	Slight decrease in crystallinity by showing broader peaks

Summary of physical/chemical characterization

[0439] Bulk stability and aqueous solubility of Compound I Form A and Compound I Amorphous Form were evaluated. The results show that they were both physically and chemically stable under various conditions. Compound I Form A and Compound I Amorphous Form exhibited comparable solubility in pH 1.2 HCl buffer, pH 4.5 50 mM acetate buffer, pH 1.6 FaSSGF, pH 5.0 FeSSGF and water; while in pH 6.8 50 mM phosphate buffer and pH 6.5 FaSSIF, the Amorphous Form showed higher solubility.

[0440] Compound II Form B is a DMSO solvate and remained unchanged after DVS test. However, racemization occurred upon heating with decreased ratio of *S*-diastereomer.

[0441] Bulk stability experiments showed that Compound II Form C was physically and chemically stable under stress conditions for 3 weeks and did not show change of color under these conditions. Compound II Form C exhibited no obvious changed in chemical purity.

[0442] Compound II fumarate salt Form B was also physically stable and did not show change of color under stress conditions and exhibited no obvious change in chemical purity. However, slight epimerization of Compound II fumarate salt Form B was observed at 60 °C.

[0443] Compound II polymorphs showed pH-dependent solubility. The solubility was overall higher at lower pH. In pH 1.2 HCl solution, pH 4.5 acetate buffer (50 mM), pH 1.6 FaSSGF, and pH 5.0 FeSSIF, all physical forms exhibited comparably good solubility. In pH 6.8 phosphate buffer (50 mM) and pH 6.5

FaSSIF, Compound II Amorphous Form and Compound II fumarate salt Form B showed better solubility than Compound II Form C. In water, only Compound II fumarate salt Form B showed good solubility, possibly due to pH shift. Slight decrease in chemical purity was observed in pH 1.2 HCl solutions for all physical forms, while the chiral purity remained unchanged.

[0444] Compound II Form C was moderately hygroscopic. It adsorbed about 3.2 wt% water from 40% RH to 80% RH at 25 °C. After the DVS test, the obtained sample remained Compound II Form C.

[0445] Compound II fumarate salt Form B was moderately hygroscopic. It adsorbed about 3.2 wt% water from 40% RH to 80% RH at 25 °C. After the DVS test, the obtained sample remained Compound II fumarate salt Form B.

[0446] Compound II Form C and Compound II fumarate salt Form B were selected as the most desirable form for downstream development based on the physical and chemical stability.

Example 7: Single crystal cultivation and analysis of Compound II

[0447] Different batches of Compound II Form B or Form C were used for single crystal cultivation.

[0448] Unless otherwise specified, Compound II Form B (prepared by the procedure described in Example 5, Preparation of Compound II Form B from Mother Liquor) and Compound II Form C (prepared by the procedure described in Example 5, Preparation of Compound II Form C (3 g scale)) were used for single crystal cultivation in Example 7.

[0449] In total, about 60 single crystal growth experiments were conducted by using a variety of solvents and different single crystal growth methods, including vapor diffusion, solvents interface diffusion, cooling, slow evaporation and temperature cycling experiments. Rod-like single crystals of Compound II Form H were obtained by vapor diffusion method using THF/MTBE solvent system. Single crystals of Compound II Form C were not obtained, possibly due to aggregation of small needle-like particles.

Approximate solubility of starting materials

[0450] Approximate solubility of Compound II Form C and Compound II Form B was conducted in previous polymorph screening studies (see Example 3). The results are used as a reference here.

Vapor diffusion experiments

[0451] Based on approximate solubility results, about 15 mg of Compound II Form C was dissolved in the minimal amount of selected solvents in a 2 mL glass vial without lid at ambient temperature (about 23-25 °C). Then the 2 mL lidless vial was placed in a 20 mL glass vial. To the 20 mL vial was added anti-solvent (some solvents contained 0.5 wt% polyvinylpyrrolidone (PVP K30)). Then the 20 mL vial was capped and placed at ambient condition for up to 6 days. Precipitates were collected and analyzed by XRPD.

Table 7-1: Crystallization by vapor diffusion Experiment 1

Solvent (mL)	Anti-solvent (mL)	XRPD	Additional analysis	Comments
DCM (0.3)	Hexane (2)	AF	//	Powder; obtained solids used for DCM/hexane experiments below
EA/DMF (1/1, v/v) (0.3)	Hexane (2)	Form C	PLM: needle-like crystals	The crystals were too small to be analyzed by SCXRD; larger solids were aggregates instead of a single crystal.
THF (0.2)	Hexane (2)	Form C	//	Aggregates; obtained solid was used for THF/EA experiments below
Water/DMF(1/1, v/v) (0.2)	Hexane (2)	//	//	Clear solution
Acetone/DMF(1/1, v/v) (0.3)	Acetone (1)	//	//	Aggregates
EA/DMF(1/1, v/v) (0.3)	EA (1)	Form C	Microscope: aggregates	Aggregates
Water/DMF(1/1, v/v) (0.4)	EA (1)	//	//	Clear solution
DCM/Hexane(8/2,v/v) (0.3)	Hexane (1)	//	//	Gel
DCM/Hexane(8/2,v/v) (0.3)	Acetone (1)	//	//	Clear solution
THF/EA(8/2,v/v) (0.4)	EA (1)	//	//	Clear solution
THF/EA(8/2,v/v) (0.4)	Heptane (1)	//	//	Aggregates
MeOH (0.5wt% PVP K30) (0.5)	Acetone (0.6)	//	//	Clear solution
MeOH (0.5wt% PVP K30) (0.5)	EA (0.6)	//	//	Clear solution
EA/DMF (1/1, v/v; 0.5wt% PVP K30) (0.25)	EA (0.4)	//	//	Hazy suspension
EA/DMF (1/1, v/v; 0.5wt% PVP K30) (0.25)	Hexane (0.2)	//	//	Aggregates

//: Not carried out

[0452] Based on approximate solubility results, about 15 mg of Compound II Form B was dissolved in the minimal amount of selected solvents in a 2 mL glass vial without lid at ambient temperature (about 23-25 °C). Then the 2 mL lidless vial was placed to a 20 mL glass vial. To the 20 mL vial was added anti-solvent.

Then the 20 mL vial was capped and placed at ambient condition for up to 10 days. Precipitates were collected and analyzed by XRPD.

Table 7-2: Crystallization by vapor diffusion Experiment 2

Solvent (mL)	Anti-solvent (mL)	XRPD	Additional analysis	Comments
Acetone (0.55)	Hexane (0.55)	//	//	Powder
Acetone (0.55)	MTBE (0.55)	//	//	Clear solution
THF (0.75)	Hexane (0.75)	//	//	Clear solution
THF (0.75)	Hexane (0.75)	Form H	PLM: rod-like to needle-like crystals	-
EA (1.2)	Acetone (1.2)	Form C	PLM: rod-like crystals	-

//: Not carried out

[0453] Compound II Form H, obtained from crystallization by vapor diffusion experiment (see Example 3, Table 3-11) was also analyzed by XRPD, PLM, and Single Crystal X-ray Diffraction (SCXRD).

Solvents interface diffusion experiments

[0454] About 20 mg of Compound II Form C was weighed into a 2 mL glass vial and a good solvent was added. After stirring at 25 °C for 10 min, suspension was filtered by a 0.45 µm syringe membrane filter. Obtained clear solution was slowly injected into an NMR tube with heavier anti-solvent in the bottom (good solvent: anti-solvent=1:1 to 1:4). Alternatively, the obtained clear solution was transferred into an empty NMR tube followed by slow injection of lighter anti-solvent on top. The NMR tubes were tightly capped and kept at room temperature.

[0455] Some clear solutions obtained from vapor diffusion experiment 1 and temperature cycling experiment were also used directly here.

Table 7-3: Solvents interface diffusion Experiment 1

Good solvent (Volume/mL)	Density of good solvent (g/cm ³)	Anti-solvent (Volume/mL)	Density of anti-solvent (g cm ⁻³)	Observation and result (after 14 days)
EtOH (0.4)	0.79	Hexane (0.8)	0.66	Tiny crystals
DCM (0.2)	1.33	Hexane (0.4)	0.66	Aggregates
Acetone (0.4)	0.79	Hexane (0.8)	0.66	Aggregates
Water/DMF (1/1, v/v) (0.3) from vapor diffusion expt. 1	1.0/0.97	EA (0.4)	0.90	Clear solution

EA (0.2)	0.90	Hexane (0.6)	0.66	Aggregates
Acetone (0.4) from temperature cycling expt	0.79	Hexane (0.6)	0.66	Aggregates
MEK (0.45) from temperature cycling expt	0.80	Hexane (0.6)	0.66	Clear solution
MeOH (0.5wt% PVP K30) (0.2)	0.79	ACN (0.4)	0.79	Clear solution
MeOH (0.5wt% PVP K30) (0.2)	0.79	MTBE (0.4)	0.74	Clear solution

[0456] About 15 mg of Compound II Form B was weighed into a 2 mL glass vial and a good solvent was added. After stirring at 25 °C for 10 min, suspension was filtered by a 0.45 µm syringe membrane filter. Obtained clear solution was slowly injected into an NMR tube with heavier anti-solvent in the bottom (good solvent: anti-solvent=1:1 to 1:4). Alternatively, the obtained clear solution was transferred into an empty NMR tube followed by slow injection of lighter anti-solvent on top. The NMR tubes were tightly capped.

Table 7-4: Solvents interface diffusion Experiment 2

Good solvent (Volume/mL)	Density of good solvent (g/cm ³)	Anti-solvent (Volume/mL)	Density of anti-solvent (g /cm ³)	Observation and result (after 10 days)
Acetone (0.55)	0.79	Water (0.55)	1.0	Aggregates
Acetone (0.55)	0.79	EA (0.55)	0.90	Clear solution
THF (0.75)	0.89	Water (0.75)	1.0	Clear solution
EA (1.2)	0.90	Water (1.2)	1.0	Clear solution

Cooling experiments

[0457] 5-20 mg of Compound II Form C was weighed into a 2 mL glass vial. Different solvent or solvent mixture (some solvents contained 0.5 wt% PVP K30) was added into the vial. Obtained mixture was stirred at 40 °C for 20 min. Then the sample was filtered by a 0.45 µm syringe membrane filter. Obtained clear solutions were kept at 40 °C for 30 min and then cooled naturally to 25 °C. If no crystal was obtained at 25 °C after 2 days, then the sample was kept at 5 °C.

[0458] Some clear solutions obtained from vapor diffusion experiment 1 and solvents interface diffusion experiment 1 were also kept at 5 °C to precipitate crystals.

Table 7-5: Cooling Experiment 1

Solvent	Volume (mL)	Observation and result (25 °C)	Observation and result (5 °C)	Comments
Acetone/DMF (1/1, v/v)	0.3	Clear solution	Clear solution	-
EA/DMF (1/1, v/v)	0.3	Clear solution	Clear solution	-
Water/DMF (1/1, v/v)	0.3	Clear solution	Clear solution	-
Acetone	0.3	Aggregates	//	Tiny needle-like crystals obtained using Form H as seed crystals
Acetone	0.2	Aggregates	Aggregates	Aggregates obtained using Form H as seed crystals
MeOH (0.5wt% PVP K30)	0.3	Clear solution	Clear solution	-
EA/DMF (1/1, v/v; 0.5wt% PVP K30)	0.3	Clear solution	Clear solution	-
Acetone/DMF (1/1, v/v) + acetone (anti-solvent)	1.4	//	Clear solution	Solution from vapor diffusion expt 1
Water/DMF + EA (anti-solvent)	1.2	//	Clear solution	Solution from vapor diffusion expt 1
DCM/Hexane (8/2, v/v) + acetone (anti-solvent)	0.3	//	Clear solution	Solution from vapor diffusion expt 1
Water/DMF (1/1, v/v) + EA (anti-solvent)	0.4	//	Clear solution	Solution obtained from interface diffusion expt 1
MEK + hexane (anti-solvent)	0.4	//	Clear solution	Solution obtained from interface diffusion expt 1
MeOH (0.5wt% PVP K30) + acetone (anti-solvent)	0.5	//	Clear solution	Solution from vapor diffusion expt 1
MeOH (0.5wt% PVP K30) + EA (anti-solvent)	0.5	//	Clear solution	Solution from vapor diffusion expt 1

EA/DMF (1/1, v/v; 0.5wt% PVP K30) + EA (anti-solvent)	0.5	//	Clear solution	Solution from vapor diffusion expt 1
MeOH (0.5wt% PVP K30) + ACN (anti- solvent)	0.5	//	Clear solution	Solution obtained from interface diffusion expt 1
MeOH (0.5wt% PVP K30) + MTBE (anti- solvent)	0.5	//	Clear solution	Solution obtained from interface diffusion expt 1

//: Not carried out

[0459] About 15 mg of Compound II Form B was weighed into a 2 mL glass vial. Different solvent or solvent mixture was added into the vial. Obtained mixtures were stirred at 25 °C for 10 min. Then the samples were stirred at 50 °C for 10 min and filtered by a 0.45 µm syringe membrane filter. Obtained clear solutions were kept at 50 °C for 30 min and then cooled to 5 °C at the rate of 0.1 °C/min. After cooling, the sample was kept at 5 °C.

Table 7-6: Cooling Experiment 2

Solvent	Volume (mL)	Observation and result (5 °C)
Acetone	0.5	Powder
EA	1	Powder

Slow evaporation experiments at room temperature

[0460] Vials containing clear solutions obtained from cooling experiment 1 were covered with a plastic lid with pin holds. The vials were placed in a fume hood at room temperature (about 23-25 °C, 20-50% RH) to allow slow evaporation of the solvents.

Table 7-7: Slow evaporation experiments at room temperature

Solvent	Observation and result (after 10 days)
EA/DMF (1/1, v/v; 0.5 wt% PVP K30)	Powder
Water/DMF (1/1, v/v) + EA	Powder
MeOH (0.5 wt% PVP K30) + acetone	Powder
MeOH (0.5 wt% PVP K30) + EA	Powder
EA/DMF (1/1, v/v; 0.5 wt% PVP K30) + EA	Aggregates
MeOH (0.5 wt% PVP K30) + MTBE	Powder

Temperature cycling experiments

[0461] About 15 mg of Compound II Form C (prepared by temperature cycle experiment in toluene/MTBE, see Table 3-4: Equilibration under a temperature cycle) was weighed into a 2 mL glass vial. Different solvent was added and the samples were incubated at 50 °C. Then the experiments were equilibrated under a temperature cycle between 5 °C and 50 °C at a heating/cooling rate of 0.1 °C/min. After 7 days, observations and results were reported.

Table 7-8: Temperature cycling experiments

Solvent	Observation and result
EA	Clear solution
Acetone	Clear solution
MEK	Clear solution

Summary of single crystal cultivation experiments

[0462] Three of the single crystals obtained from the same bulk sample of Compound II Form H in THF/MTBE vapor diffusion experiments were used for single crystal analysis, named as SCXRD-01, SCXRD-02 and SCXRD-03.

[0463] Based on single crystal data of SCXRD-01 (*cu_2023034_0m_sq*), the single crystal is crystallized in trigonal system, $P3_1$ space group with $R_{int}=10.7\%$, the final $R1=[I>2\sigma(I)]=7\%$ at 100K and Flack parameter=0.10(19). 1/3 equivalent of THF molecule is detected in each crystal cell. The Ortep image of Compound II Form C SCXRD-01 is shown in FIG. 45, which confirms the absolute configuration of ‘S,S,S’. Chirality of carbon atoms C10, C11 and C24 is determined by priority rules.

[0464] Based on single crystal data of SCXRD-02 (*cu_2023126_0m*), the single crystal is crystallized in trigonal system, $P3_1$ space group with $R_{int}=9.9\%$, the final $R1=[I>2\sigma(I)]=8.2\%$ at 100K and Flack parameter=0.11(9). 2/3 equivalent of MTBE molecule is detected in each crystal cell. The Ortep image of Compound II Form C SCXRD-02 is shown in FIG. 46, which confirms the absolute configuration of ‘S,S,S’. Chirality of carbon atoms C10, C15 and C24 are determined by priority rules.

[0465] Based on single crystal data of SCXRD-03 (*cu_2023129_0m*), the single crystal is crystallized in trigonal system, $P3_1$ space group with $R_{int}=6.1\%$, the final $R1=[I>2\sigma(I)]=11.0\%$ at 100K and Flack parameter=0.16(4). 2/3 equivalent of THF molecule and 1/3 equivalent of MTBE molecule are detected in each crystal cell. The Ortep image of Compound II Form C SCXRD-03 is shown in FIG. 47, which confirms the absolute configuration of ‘S,S,S’. Chirality of carbon atoms C10, C15 and C23 are determined by priority rules.

[0466] The SCXRD data for experiments SCXRD-01, 02, and 03 are shown in Table 7-9.

Table 7-9: Single crystal data

SCXRD-01 (<i>cu_2023034_0m_sq</i>)	
$3(\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_4) \cdot \text{C}_4\text{H}_8\text{O}$	$D_x = 1.132 \text{ Mg m}^{-3}$
$M_r = 1492.77$	Cu $K\alpha$ radiation, $\lambda = 1.54178 \text{ \AA}$
Trigonal, $P3_1$	Cell parameters from 8405 reflections
$a = 24.1274 (6) \text{ \AA}$	$\alpha = 3.7\text{--}74.1^\circ$
$b = 24.1274 (6) \text{ \AA}$	$\beta = 0.62 \text{ mm}^{-1}$
$c = 13.0308 (4) \text{ \AA}$	$T = 100 \text{ K}$
$V = 6569.4 (4) \text{ \AA}^3$	Needle, colourless
$Z = 3$	$0.12 \times 0.08 \times 0.05 \text{ mm}$
$F(000) = 2388$	
SCXRD-02 (<i>cu_2023126_0m</i>)	
$3(\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_4) \cdot 2(\text{C}_5\text{H}_{12}\text{O})$	$D_x = 1.191 \text{ Mg m}^{-3}$
$M_r = 1596.96$	Cu $K\alpha$ radiation, $\lambda = 1.54178 \text{ \AA}$
Trigonal, $P3_1$	Cell parameters from 9878 reflections
$a = 24.2881 (14) \text{ \AA}$	$\alpha = 3.6\text{--}74.3^\circ$
$b = 24.2881 (14) \text{ \AA}$	$\beta = 0.64 \text{ mm}^{-1}$
$c = 13.0707 (12) \text{ \AA}$	$T = 170 \text{ K}$
$V = 6677.5 (10) \text{ \AA}^3$	Block, colourless
$Z = 3$	$0.16 \times 0.08 \times 0.05 \text{ mm}$
$F(000) = 2568$	
SCXRD-03 (<i>cu_2023129_0m</i>)	
$3(\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_4) \cdot 2(\text{C}_4\text{H}_8\text{O}) \cdot \text{C}_5\text{H}_{12}\text{O}$	$D_x = 1.236 \text{ Mg m}^{-3}$
$M_r = 1653.02$	Cu $K\alpha$ radiation, $\lambda = 1.54178 \text{ \AA}$
Trigonal, $P3_1$	Cell parameters from 9844 reflections
$a = 24.2722 (5) \text{ \AA}$	$\alpha = 3.6\text{--}79.8^\circ$
$b = 24.2722 (5) \text{ \AA}$	$\beta = 0.67 \text{ mm}^{-1}$
$c = 13.0585 (4) \text{ \AA}$	$T = 100 \text{ K}$
$V = 6662.6 (3) \text{ \AA}^3$	Block, colourless
$Z = 3$	$0.16 \times 0.08 \times 0.05 \text{ mm}$
$F(000) = 2658$	

[0467] Analysis of three single crystal structures shows that the inter-molecular interaction is weak, and only one H-bond (3-coordinate N on 4-ring as acceptor and imide N-H on 6-ring as donor) exists. Compound II Form H molecules are stacked in the direction of the *c*-axis through hydrogen bonding, and

then arranged in the *a*-axis and *b*-axis direction through van der Waals forces; the side-by-side channels are filled with THF and/or MTBE molecules. Due to similar cell parameters, the three single crystals show similar simulated XRPD patterns with different solvent level. Since the single crystals belong to different solvates, simulated pattern is not consistent with the measured XRPD of Compound II Form H and Compound II Form H could be mixed phases. The single crystal of SCXRD-03, after SCXRD analysis, was recovered and measured by UPCC. The crystal of SCXRD-03 shows one single peak corresponding to the 'S,S,S-isomer.

Example 8: Rat PK studies of Compound I and Compound II

[0468] The pharmacokinetic properties of Compound I and Compound II were determined using the protocol described below.

[0469] Compound I Form A, Compound I Amorphous Form, Compound II Form C, and Compound II fumarate salt Form B were selected for testing in rat PK studies. Compound I Form A was prepared by the procedure described in Example 1, Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione. Compound I Amorphous Form was prepared by the procedure described in Example 5, Preparation of Compound I Amorphous Form (1.2 g scale).

[0470] Compound II Amorphous Form prepared by the procedure described in Example 3, Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione. Compound II fumarate salt Form B was prepared by the procedure described in Example 5, Preparation of Compound II fumarate salt Form B (800 mg scale).

[0471] For the rat PK studies, a total of three female Sprague Dawley rats each were used for each dosage. Table 8-1 below summarizes the formulations that were administered via oral gavage (PO) or single bolus IV.

Table 8-1: Formulations for Rat PK Testing

Polymorph	Dosage (mg/kg)	Conc. (mg/mL)	Vehicle*	Dosage Route
Compound I Form A	50	5	0.5% MC (4000 cps) and 0.4% Tween80 in water	PO
	150	15		PO
	500	50		PO
Compound I Amorphous Form	50	5	0.5% MC (4000 cps) and 0.4% Tween80 in water	PO
	150	15		PO
	500	50		PO
Compound II Form C	10	2	5% DMSO , 5% solutol , 90% water	IV bolus
	30	3		PO

	100	10	0.5% MC (4000 cps) and 0.4% Tween80 in water	PO
Compound II fumarate salt Form B	10	2	5% DMSO , 5% solutol , 90% water	IV bolus
	30	3	0.5% MC (4000 cps) and 0.4% Tween80 in water	PO
	100	10		PO

*MC: methyl cellulose; Tween80: Polyethylene glycol sorbitan monooleate

[0472] Samples (approximately 0.3 mL per timepoint) were collected at 0.25 hr, 0.5 hr, 1 hr, 2 hrs, 4 hrs, 8 hrs, and 24 hrs in tubes containing EDTA-K2 as anti-coagulant and placed on ice before centrifuging. Blood samples were processed for plasma by centrifugation at approximately 4 °C, 3,200 g for 10 min within 0.5 hrs of collection. Plasma was collected respectively and transferred into pre-labeled 96 well plate or polypropylene tubes with 2 M citric acid as stabilizer (5 uL citric acid was added into 95 uL plasma) on wet ice at each time point, mixed well, quick frozen over dry ice, and kept at -60 °C or lower until LC-MS/MS analysis.

[0473] Plasma drug concentrations were determined by LC/MS/MS using standard curve calibration. Plasma concentration versus time data was plotted in graph and analyzed by non-compartmental approaches using the Phoenix WinNonlin 6.3 software program. Related PK parameters were calculated according to dosing route, e.g. Cl, Vdss and C₀ for intravenous administration, C_{max}, T_{max} or %F for extravascular administration, and T_{1/2}, AUC_(0-t), AUC_(0-inf), MRT_(0-t), MRT_(0-inf) for all routes. Table 8-2 summarizes the AUC and C_{max} data for the tested compounds.

Table 8-2: Pharmacokinetic data for Compound I and Compound II

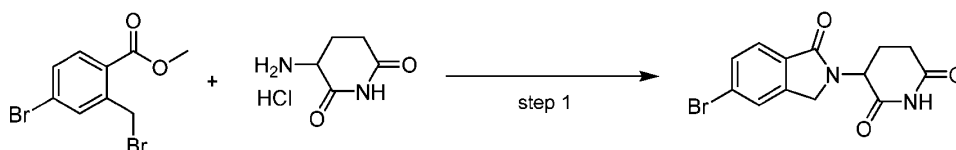
Study	Polymorph	Dosage (mg/kg)	Dosage Route	C _{max} (ng/mL)	AUC (hr·ng/mL)
1	Compound I Form A	50	Oral	8968	47335
		150	Oral	16389	158738
		500	Oral	30732	641000
2	Compound I Amorphous Form	50	Oral	2696	12453
		150	Oral	7073	60013
		500	Oral	11695	255589
3	Compound II Form C	10	IV bolus	5671	6260
		30	Oral	2792	10504
		100	Oral	11748	85247
4	Compound II fumarate salt Form B	10	IV bolus	6298	6560
		30	Oral	2525	10884
		100	Oral	8452	62551

[0474] Compound I Form A exhibited higher bioavailability compared to Compound I Amorphous Form at all tested dosages. Compound II Form C showed slightly higher bioavailability than Compound II fumarate salt Form B when administered orally.

Example 9: Alternative Synthesis of Compound I and Compound II

Procedure A: Synthesis of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I)

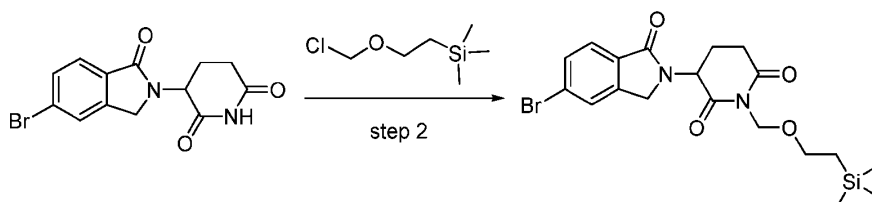
Step 1:



[0475] **3-(5-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione:** To a solution of methyl 4-bromo-2-(bromomethyl)benzoate (50 g, 162.36 mmol, 1 eq) and 3-aminopiperidine-2,6-dione hydrochloride (32.07 g, 194.83 mmol, 1.2 eq) in DMF (500 mL) was added DIEA (83.93 g, 649.42 mmol, 113.12 mL, 4 eq) at 20 °C. The mixture was stirred 120 °C for 12 hr. LCMS showed the starting material was consumed completely. The mixture was filtered and the filtrate was concentrated under vacuum to give the title compound (42 g, 80% yield) as a pink solid, which was used in the next step without further purification.

[0476] ¹H NMR (400 MHz, DMSO-*d*₆) δ = 11.00 (s, 1H), 7.89 (s, 1H), 7.74 - 7.63 (m, 2H), 5.10 (dd, *J* = 5.1, 13.3 Hz, 1H), 4.52 - 4.28 (m, 2H), 2.96 - 2.85 (m, 1H), 2.59 (br d, *J* = 17.4 Hz, 1H), 2.38 (dq, *J* = 4.4, 13.2 Hz, 1H), 2.08 - 1.92 (m, 1H).

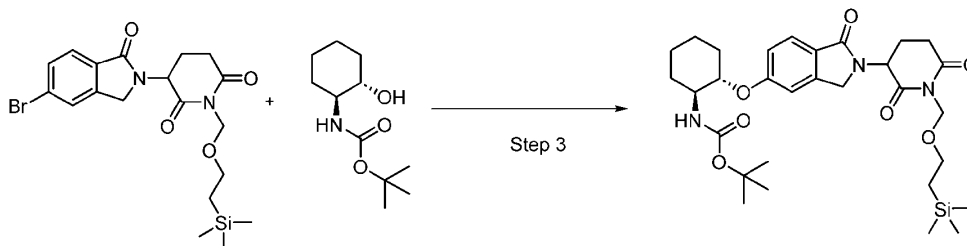
Step 2:



[0477] **3-(5-bromo-1-oxoisoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione:** To a solution of 3-(5-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (80 g, 247.57 mmol, 1 eq) and DBU (75.38 g, 495.14 mmol, 2 eq) in DMF (800 mL) was added SEM-Cl (61.91 g, 371.35 mmol, 1.5 eq) dropwise at 0 °C. The mixture was stirred at 20 °C for 5 h. TLC showed the reaction was complete. The mixture was poured into water (1 L) and extracted with MTBE (3 x 500 mL). The combined organic phases were washed with brine (3 x 200 mL), dried over Na₂SO₄ and concentrated under vacuum. The residue was purified by column chromatography on silica gel, eluted with (petroleum ether: ethyl acetate = 10:1 to 1:1) to give the title compound (75 g, yield 66.8%) as a white solid.

[0478] ¹H NMR (400 MHz, CDCl₃) δ = 7.76 (d, *J* = 8.6 Hz, 1H), 7.68 - 7.60 (m, 2H), 5.28 - 5.16 (m, 3H), 4.52 - 4.28 (m, 2H), 3.68 - 3.56 (m, 2H), 3.08 - 3.00 (m, 1H), 2.96 - 2.84 (m, 1H), 2.40 - 2.28 (m, 1H), 2.24 - 2.16 (m, 1H), 0.96 - 0.92 (m, 2H), 0.01 (s, 9H).

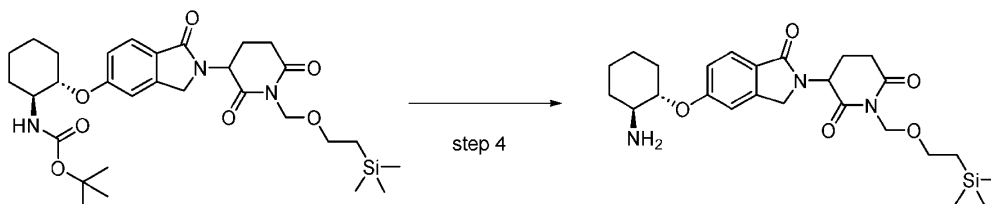
Step 3:



[0479] *tert-butyl ((1S,2S)-2-((2-(2,6-dioxo-1-((2-(trimethylsilyl)ethoxy)methyl)piperidin-3-yl)-1-oxoisindolin-5-yl)oxy)cyclohexyl)carbamate*: To a mixture of 3-(5-bromo-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione (2 g, 4.41 mmol, 1 eq), *tert-butyl ((1S,2S)-2-hydroxycyclohexyl)carbamate* (1.42 g, 6.62 mmol, 1.5 eq), 4-*tert-butyl*-2-(4-*tert-butyl*-2-pyridyl)pyridine (59 mg, 0.22 mmol, 0.05 eq), Ir[(dF(CF₃)ppy)₂dtbbpy]PF₆ (49 mg, 44.1 μmol, 0.01 eq) and NiCl₂(glyme) (49 mg, 0.22 mmol, 0.05 eq) in MeCN (15 mL) was added 2,2,6,6-tetramethylpiperidine (623 mg, 4.41 mmol, 1 eq). The mixture was stirred for 12 h under irradiation of blue LED light at 20°C. LCMS showed the reaction was completed. The mixture was filtered and filtrate was concentrated under vacuum. The residue was purified by column chromatography on silica gel eluted with petroleum ether: ethyl acetate (10:1 to 1:3) to provide the title compound (1.94 g, yield 75%) as a yellow solid.

[0480] ¹H NMR (400 MHz, DMSO-d₆) δ = 7.60 (dd, *J* = 1.9, 8.4 Hz, 1H), 7.19 - 7.11 (m, 1H), 7.07 - 6.99 (m, 1H), 6.87 - 6.79 (m, 1H), 5.26 - 5.14 (m, 1H), 5.04 (q, *J* = 10.0 Hz, 2H), 4.46 - 4.38 (m, 1H), 4.24 - 4.17 (m, 2H), 3.56 - 3.49 (m, 2H), 3.16 - 3.03 (m, 2H), 2.82 - 2.73 (m, 1H), 2.44 - 2.34 (m, 1H), 2.16 - 2.01 (m, 2H), 1.85 - 1.76 (m, 2H), 1.69 - 1.54 (m, 3H), 1.30 (s, 9H), 1.14 - 1.08 (m, 2H), 0.89 - 0.79 (m, 2H), -0.02 (d, *J* = 0.9 Hz, 9H).

Step 4:

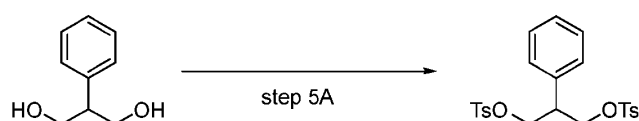


[0481] *3-(5-(((1S,2S)-2-aminocyclohexyl)oxy))-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione*: To a solution of *tert-butyl ((1S,2S)-2-((2-(2,6-dioxo-1-((2-(trimethylsilyl)ethoxy)methyl)piperidin-3-yl)-1-oxoisindolin-5-yl)oxy)cyclohexyl)carbamate* (2 g, 3.4 mmol, 1 eq) in DCM (15 mL) was added 2,6-lutidine (911 mg, 8.51 mmol, 2.5 eq) and TMSOTf (1.51 g,

6.81 mmol, 2 eq). The resulting mixture was stirred at 25°C for 24 h. LCMS showed the reaction was completed. The reaction mixture was concentrated under vacuum. The residue was purified by reversed phase column (0.1% TFA) to give the title compound (1.08 g, yield 65%) as yellow solid.

[0482] ¹H NMR (400 MHz, DMSO-d₆) δ = 8.04 (br s, 3H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.27 (dd, *J* = 1.7, 5.9 Hz, 1H), 7.15 (td, *J* = 2.7, 8.3 Hz, 1H), 5.19 (dd, *J* = 4.6, 13.2 Hz, 1H), 5.05 (q, *J* = 9.7 Hz, 2H), 4.50 - 4.20 (m, 3H), 3.58 - 3.46 (m, 2H), 3.29 (br d, *J* = 9.0 Hz, 2H), 3.14 - 2.97 (m, 1H), 2.84 - 2.71 (m, 1H), 2.45 - 2.31 (m, 1H), 2.25 - 2.14 (m, 1H), 2.05 (br dd, *J* = 2.4, 5.4 Hz, 1H), 1.72 (br d, *J* = 11.0 Hz, 2H), 1.44 - 1.29 (m, 4H), 0.90 - 0.78 (m, 2H), 0.05 - 0.08 (m, 9H).

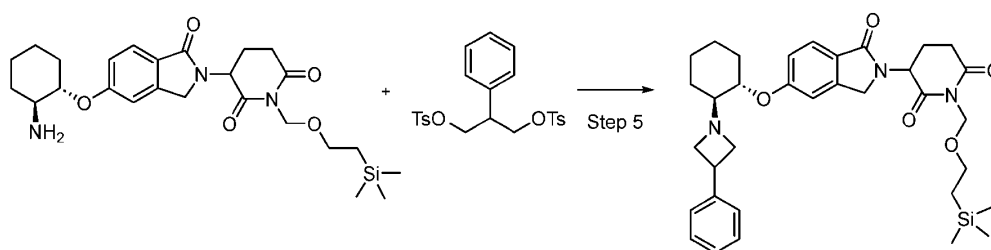
Step 5A:



[0483] **2-phenylpropane-1,3-diyl bis(4-methylbenzenesulfonate)**: To a solution of 2-phenylpropane-1,3-diol (5 g, 32.85 mmol, 1 eq) in DCM (100 mL) was added TsCl (21.92 g, 114.99 mmol, 3.5 eq), DMAP (401.37 mg, 3.29 mmol, 0.1 eq) and Et₃N (13.30 g, 131.41 mmol, 18.29 mL, 4 eq), the mixture was stirred at 20°C for 16 hr. TLC showed the reaction was completed. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (Petroleum ether/Ethyl acetate=100:1, 50:1) to afford 2-phenylpropane-1,3-diyl bis(4-methylbenzenesulfonate) (6.5 g, 12.70 mmol, 38.66% yield, 90% purity) as white solid.

[0484] ¹H NMR (400 MHz, chloroform-*d*) δ ppm 2.45 (s, 6 H) 3.27 (m, 1 H) 4.21 (d, *J*=6.02 Hz, 4 H) 7.01 - 7.07 (m, 2 H) 7.21 - 7.27 (m, 3 H) 7.30 (d, *J*=8.03 Hz, 4 H) 7.67 (d, *J*=8.53 Hz, 4 H).

Step 5:

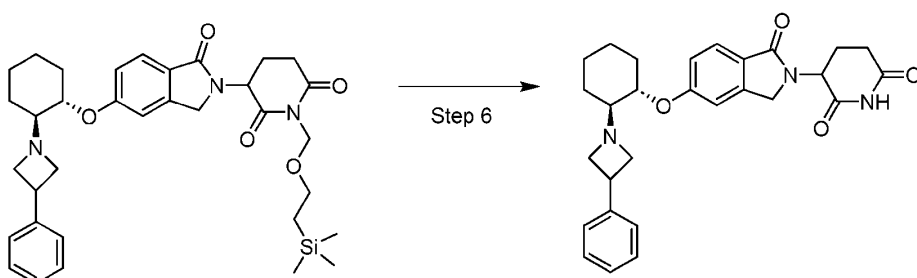


[0485] **3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione**: 3-(5-(((1*S*,2*S*)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione (2 g, 4.1 mmol, 1 eq), 2-phenylpropane-1,3-diyl bis(4-methylbenzenesulfonate) (2.27 g, 4.92 mmol, 1.2 eq) and DIEA (2.12 g, 16.4 mmol, 4 eq) were taken up into a microwave tube in MeCN (10 mL). The sealed tube was heated at 120°C and stirred for 16 h. LCMS showed the reaction was completed. The reaction mixture was concentrated

under vacuum. The residue was purified by reversed phase column (0.5% TFA) and SFC separation to give the title compound (0.9 g, 36% yield) as a white solid.

[0486] ¹H NMR (400 MHz, DMSO-d₆) δ = 7.61 (d, *J* = 8.4 Hz, 1H), 7.34 - 7.26 (m, 4H), 7.22 - 7.16 (m, 2H), 7.05 (dd, *J* = 2.1, 8.5 Hz, 1H), 5.17 (dd, *J* = 5.1, 13.4 Hz, 1H), 5.10 - 4.99 (m, 2H), 4.44 - 4.15 (m, 3H), 3.75 - 3.59 (m, 2H), 3.55 - 3.46 (m, 3H), 3.27 (br d, *J* = 8.2 Hz, 2H), 3.18 - 2.98 (m, 2H), 2.83 - 2.73 (m, 1H), 2.43 - 2.36 (m, 1H), 2.04 - 2.00 (m, 1H), 1.84 (br dd, *J* = 2.3, 10.2 Hz, 1H), 1.65 (br d, *J* = 5.0 Hz, 2H), 1.39 (br t, *J* = 8.5 Hz, 2H), 1.30 - 1.22 (m, 2H), 1.17 - 1.10 (m, 1H), 0.89 - 0.77 (m, 3H), -0.03 (d, *J* = 3.8 Hz, 8H).

Step 6:



[0487] **3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione:** To a solution of 3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione (22 g, 36.5 mmol, 1 *eq*) in DCM (200 mL) was added MsOH (14.01 g, 145.82 mmol, 10.38 mL, 4 *eq*) at 20°C. The mixture was stirred for 2 h at 20°C. N1,N2-dimethylethane-1,2-diamine (3.85 g, 43.69 mmol, 2.31 mL, 1.2 *eq*) and TEA (29.49 g, 291.34 mmol, 40.54 mL, 8 *eq*) was added to above mixture at 20°C. The mixture was stirred at 20°C for 2 h. LCMS showed the starting material was consumed completely. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The residue was purified by Prep-HPLC (neutral) to give the title compound (10.02 g, 58.07% yield) as a white solid.

[0488] ¹H NMR (400 MHz, DMSO-d₆) δ = 10.96 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.38 - 7.25 (m, 4H), 7.23 - 7.14 (m, 2H), 7.04 (br d, *J* = 8.4 Hz, 1H), 5.06 (dd, *J* = 4.9, 13.3 Hz, 1H), 4.45 - 4.17 (m, 3H), 3.82 - 3.46 (m, 3H), 3.30 - 3.04 (m, 2H), 2.96 - 2.82 (m, 1H), 2.59 (br d, *J* = 17.0 Hz, 1H), 2.48 - 2.27 (m, 2H), 2.09 - 1.92 (m, 2H), 1.92 - 1.80 (m, 1H), 1.66 (br s, 2H), 1.45 - 1.33 (m, 2H), 1.32 - 1.21 (m, 1H), 1.20 - 1.07 (m, 1H).

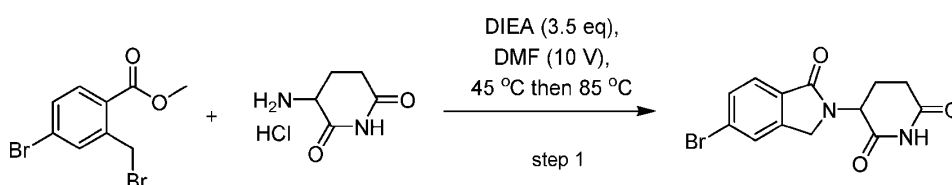
[0489] The two isomers ((*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione and (*R*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione) from Procedure A can be separated as described herein or the literature to yield Compound II, (*S*)-3-

(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione, as a single stereoisomer.

[0490] Alternatively, Compound II was provided without the need for a final separation step using Procedure B. In Procedure B, each of the intermediates was confirmed using standard characterization techniques (e.g., HPLC) as compared to a reference standard (e.g., the compounds produced in the reactions described herein or the literature).

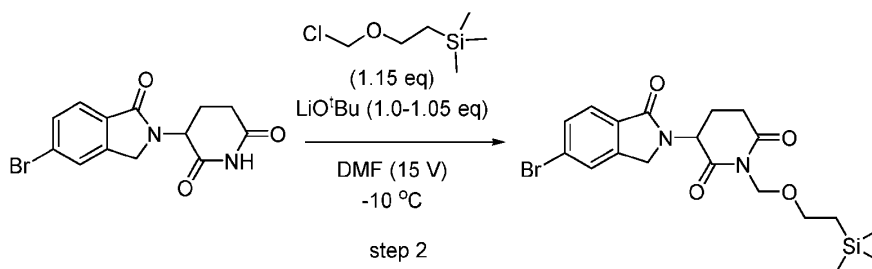
Procedure B: Synthesis of (*S*)-3-(1-oxo-5-(((1*S*,2*S*)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II)

Step 1:



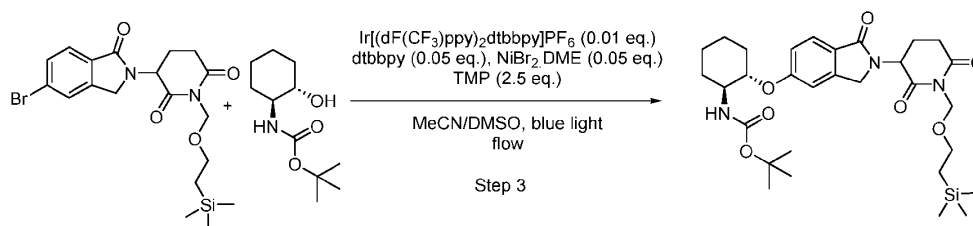
[0491] **3-(5-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione:** To a solution of methyl 4-bromo-2-(bromomethyl)benzoate and 3-aminopiperidine-2,6-dione hydrochloride in DMF (10 V) was added DIEA (3.5 eq). The reaction mixture was heated to 45 °C for 22 hours and then the temperature increased to 85 °C for an additional 21 hours. After the reaction was determined complete (LCMS showed the starting material was consumed), water was added and the precipitate was isolated. After crystallization, the resulting product was isolated with 99.6% purity in 84% isolated yield.

Step 2:



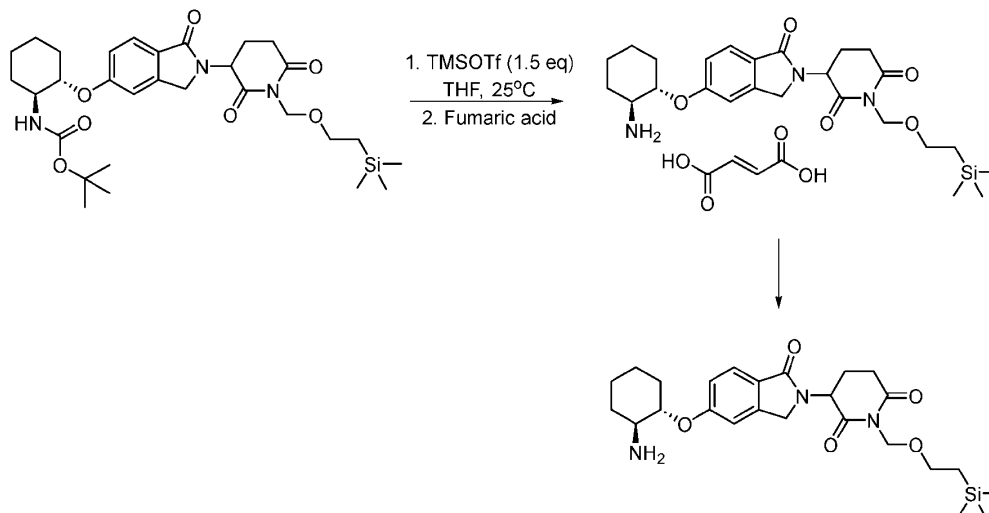
[0492] **3-(5-bromo-1-oxoisoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione:** To a solution of 3-(5-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione in DMF (15V) at -10 °C was added *t*-BuOLi (1-1.5 eq), and the mixture was stirred for 6 hours at -10 °C. SEM-Cl (1.15 eq) was added at -10 °C and the mixture was stirred while allowing to warm to 25 °C until reaction completion, approx. 1-3 hours. After reaction completion, water was added and mixture cooled to 10 °C and stirred for approximately 10-20 hours the desired product was obtained via filtration in 97.6% purity and 88% isolated yield.

Step 3:



[0493] *tert*-butyl ((1*S*,2*S*)-2-((2-(2,6-dioxo-1-((2-(trimethylsilyl)ethoxy)methyl)piperidin-3-yl)-1-oxoisindolin-5-yl)oxy)cyclohexyl)carbamate: A mixture of 3-(5-bromo-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione, *tert*-butyl ((1*S*,2*S*)-2-hydroxycyclohexyl)carbamate (1.5 eq), 4-*tert*-butyl-2-(4-*tert*-butyl-2-pyridyl)pyridine (0.05 eq), Ir[(dF(CF₃)ppy)₂dtbbpy]PF₆ (0.01 eq) and NiCl₂.DME (0.05 eq) in MeCN (20V) / DMSO (4.5V) was reacted at 40 °C using a flow mode photochemical reaction under blue light at 40 °C for 120 min of the residence time in the single-layer reactor of 1/4 inch FEP fluid tube. A telescoped work-up approach was used to obtain a THF solution of the desired product, which was directly carried forward for the next step. After reaction completion, the THF solution containing the desired product was obtained in two batches in 58% assay yield (61% assay yield for the first batch and 53% assay yield for the second batch). After purification, a THF solution of *tert*-butyl ((1*S*,2*S*)-2-((2-(2,6-dioxo-1-((2-(trimethylsilyl)ethoxy)methyl)piperidin-3-yl)-1-oxoisindolin-5-yl)oxy)cyclohexyl)carbamate was obtained with 72.8% purity in about 61% yield.

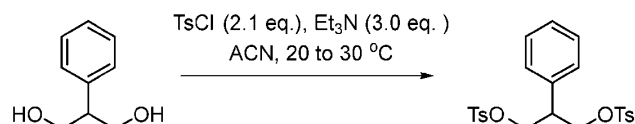
Step 4:



[0494] 3-(5-(((1*S*,2*S*)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione: To a solution of *tert*-butyl ((1*S*,2*S*)-2-((2-(2,6-dioxo-1-((2-(trimethylsilyl)ethoxy)methyl)piperidin-3-yl)-1-oxoisindolin-5-yl)oxy)cyclohexyl)carbamate in THF (10V) was added TMSOTf (1.2 eq) and the reaction was stirred at 25 °C for an hour, and an additional 0.3

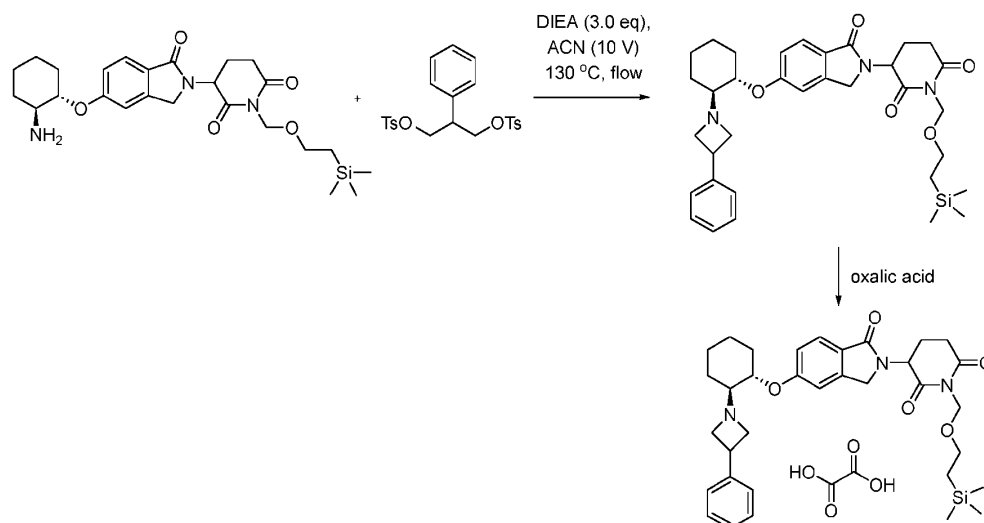
eq of TMSOTF was added, and the reaction was stirred an additional 16 hours. The crude reaction was extracted with ethyl acetate and washed with water. The crude product in ethyl acetate was stirred at 50 °C and was treated with 1 eq. fumaric acid and stirred for 7 hr at 50 °C. The mixture was cooled to RT and n-heptane was added to produce a slurry which was filtered to provide 3-(5-(((1S,2S)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione fumarate. The resulting 3-(5-(((1S,2S)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione fumarate was resuspended in EtOAc and stirred for 1 hour at 25 °C. After 1 hour, 7% aq NaHCO₃ was added, and the resulting aq phase pH was 7.5. Water was added and the organic phase was separated. ACN was added to the resulting ethyl acetate solution, and the organic solvents were removed under vacuum and mild heat, this was repeated several times to ultimately provide 3-(5-(((1S,2S)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione a solution in ACN in 96.8% purity and 74% yield.

Step 5A:



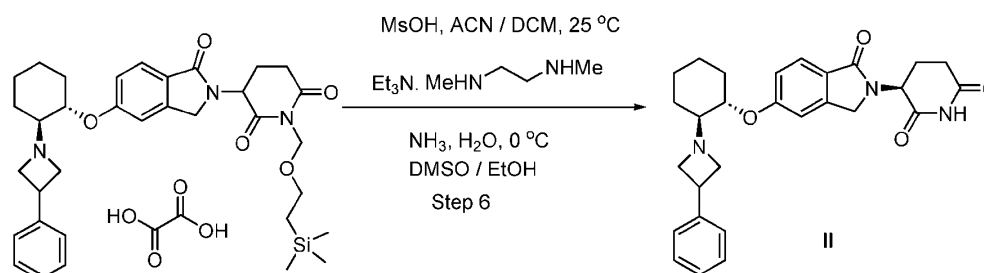
[0495] 2-phenylpropane-1,3-diyl bis(4-methylbenzenesulfonate): 2-phenylpropane-1,3-diol was reacted with tosyl-chloride (2.1 eq) and triethyl amine (3 eq) in acetonitrile at 20-30 °C to provide the desired product. Utilizing acetonitrile allowed for lower reagent ratios and improved scaled purification as compared to literature procedure. Direct crystallization with the addition of water into the reaction mixture afforded 2-phenylpropane-1,3-diyl bis(4-methylbenzenesulfonate). After work up and isolation, 2-phenylpropane-1,3-diyl bis(4-methylbenzenesulfonate) was obtained in 99.9% purity and 92% isolated yield.

Step 5:



[0496] 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione oxalate: 3-(5-(((1S,2S)-2-aminocyclohexyl)oxy)-1-oxoisindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione, 2-phenylpropane-1,3-diyl bis(4-methylbenzenesulfonate), and DIEA (3 eq) were combined in ACN (10 V) at 130 °C under flow chemistry conditions for a residence time of 2 hours. The crude mixture was cooled to 25 °C and 0.6 equivalents of di-tert-butyl-dicarbonate was added and the mixture was stirred for 16 hours. The mixture was then charged with 10 vol. 10% KH_2PO_4 , the wet cake was filtered and rinsed with acetonitrile and water and extracted into isopropyl acetate. To this solution of 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione in isopropylacetate was added 1.05 eq oxalic acid to the solution, which provided the desired product in 98.7% purity and 26% isolated yield.

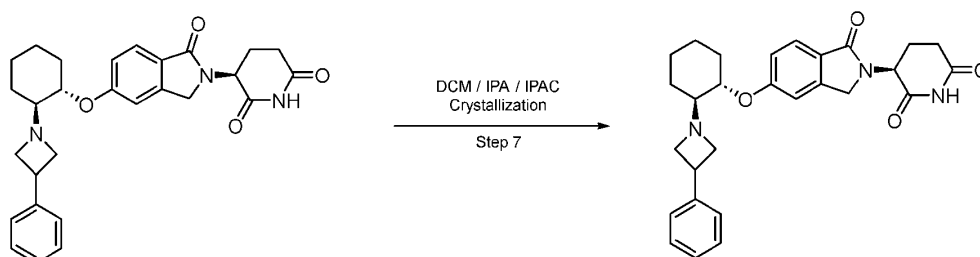
Step 6:



[0497] (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione: To a solution of 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione oxalate in 10 V ACN/DCM (1:2 ratio) at 25 °C was added 4.7 eq. MsOH and the mixture was stirred at 25 °C. After 24 hours, N1,N2-dimethylethane-1,2-

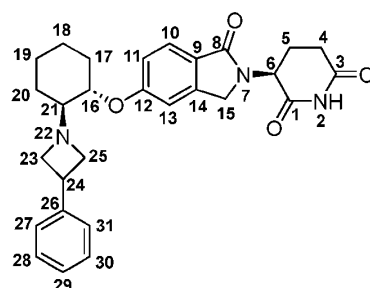
diamine (1.5 *eq*) and TEA (10 *eq*) were added, NH₃ in water was added and the mixture was stirred at 40 °C for 30 hours. After aqueous quenching and work-up with 10 % KH₂PO₄ solution and adjustment of the pH to pH=6. Crude product was extracted the aqueous with DCM, and then solvent was evaporated and replaced with EtOH and this process was repeated to provide a crude slurry in EtOH. The slurry was further dried and filtered, and rinsed with DMSO to provide enrichment of the desired isomer of (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione in 99.8% purity and R,SS/S,SS = 5.0%/95.0% in 69% isolated yield.

Step 7:



[0498] Recrystallized (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound II) was obtained from the crude (S)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione from Step 6 by washing with a 10% Na₂SO₄ solution, concentrating with DCM, and crystallization in a DCM (6.6 V) / IPA (3.3 V) / IPAC (20 V) system to yield the desired product with 99.9% purity, R,SS/S,SS=3.9%/96%, as Form C in 84% isolated yield.

[0499] ¹H and ¹³C NMR data provided was generated in MeOD-*d*₄ at 27 °C using a Bruker AVANCE III 400 MHz NMR spectrometer equipped with a Bruker 5 mm PABBO BB-400 MHz Z-gradient high resolution probe, and supported by TOPSPIN software. The atom numbers referred to in the table are as shown below.



Atom Number	Type	δ_H (ppm) (mult, <i>J</i> in Hz)	δ_C (ppm) (mult, <i>J</i> in Hz)	Relative Intensity
1	C=O	N/A	172.30	N/A
2	NH	N/A	N/A	N/A

Atom Number	Type	δ_H (ppm) (mult, J in Hz)	δ_C (ppm) (mult, J in Hz)	Relative Intensity
3	C=O	N/A	174.71	N/A
4a	CH ₂	2.90 (m)	32.39	1H
4b		2.77 (m)		1H
5a	CH ₂	2.47 (ddd, J = 18.0, 13.3, 4.8 Hz)	24.14	1H
5b		2.15 (ov)		1H
6	CH	5.11 (dd, J = 13.3, 5.2 Hz)	53.60	1H
7	N	N/A	N/A	N/A
8	C=O	N/A	171.45	N/A
9	C	N/A	125.10	N/A
10	CH	7.71 (d, J =8.4 Hz)	125.99	1H
11	CH	7.06 (dd, J =8.4, 2.1 Hz)	117.57	1H
12	C	N/A	162.64	N/A
13	CH	7.14 (d, J =1.7 Hz)	110.32	1H
14	C	N/A	146.31	N/A
15a	CH ₂	4.46 (d, J =17.0 Hz)	48.79	1H
15b		4.40 (d, J =17.0 Hz)		1H
16	CH	4.32 (m)	82.62	1H
17a	CH ₂	2.17 (ov)	30.85	1H
17b		1.41 (ov)		1H
18a	CH ₂	1.78 (ov)	25.02 or 24.87	1H
18b		1.46 (ov)		1H
19a	CH ₂	1.78 (ov)	25.02 or 24.87	1H
19b		1.46 (ov)		1H
20a	CH ₂	2.01 (m)	28.63	1H
20b		1.15 (m)		1H
21	CH	2.58 (m)	69.95	1H
22	N	N/A	N/A	N/A
23a/25a		3.80 (t, J =7.8 Hz)		2H
23b/25b	CH ₂	3.53 (t, J =8.0 Hz) 3.33 (ov)	63.95, 60.73	1H 1H
24	CH	3.67 (m)	37.46	1H

Atom Number	Type	δ_H (ppm) (mult, J in Hz)	δ_C (ppm) (mult, J in Hz)	Relative Intensity
26	C	N/A	142.85	N/A
27/31	CH	7.27 (ov)	127.92	2H
28/30	CH	7.30 (ov)	129.50	2H
29	CH	7.19 (m)	127.60	1H

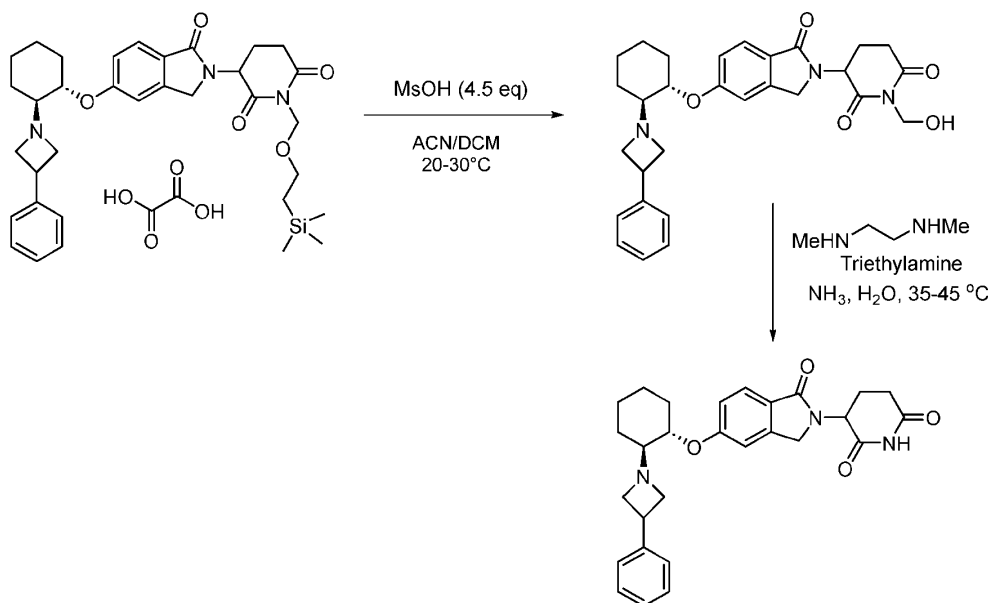
[0500] *HRMS*: High resolution mass spectrometric analysis was performed on an Agilent G6224A TOF LC/MS using high performance liquid chromatography with electrospray ionization time of flight mass spectrometry (HPLC-ESI-TOF/MS). Electrospray ionization was performed in positive ion mode. The solution for analysis was prepared by dissolving 2.633 mg of Compound II in 30 mL of ACN. The sample yielded a characteristic positive ion with $[M+H]^+$ observed at 474.2399 m/z corresponding to protonated Compound II $C_{28}H_{32}N_3O_4^+$ (calculated 474.2387 m/z).

[0501] *Elemental Analysis*: Carbon, hydrogen, nitrogen analysis of Compound II was performed on a Thermo Flashsmart Elemental Analyzer. The sample was analyzed in duplicate. Anal. Calcd for $C_{28}H_{31}N_3O_4$: C, 71.02; H, 6.60; N, 8.87. Found: C, 71.28; H, 6.63; N, 8.86.

[0502] *Optical Rotation*: The optical rotation analysis for Compound II was performed using Rudolph Autopol V Automatic Polarimeter instrument under 25 °C. A 99.20 mg sample of Compound II was weighed and added into a 10 mL volumetric flask, dissolved in pyridine, and diluted to volume with pyridine. The solvent blank was measured followed by the sample solution, providing a $[\alpha]_D^{25}$ of +17.3° (calculated on the basis). Analysis parameters are as below.

Item	Parameters
Temperature (°C)	25°C
Wavelength (nm)	589
Solvent	pyridine
Length of polarimeter tube (dm)	1

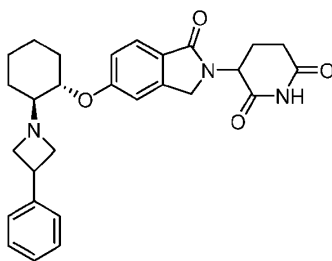
Procedure C: Synthesis of 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione (Compound I)



[0001] 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione as a 1:1 mixture of mixed isomers: To a solution of 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)piperidine-2,6-dione oxalate was added MsOH (4.5 eq) in ACN/DCM at 20-30 °C to provide 1-(hydroxymethyl)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione which is typically not isolated from the reaction mixture but observed as an intermediate. To the resulting 1-(hydroxymethyl)-3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione was added N1,N2-dimethylethane-1,2-diamine (1.5 eq) and TEA (10 eq), followed by $\text{NH}_3 \cdot \text{H}_2\text{O}$ in DCM/ACN and the mixture was stirred at 35 to 45 °C for 30 hours. After aqueous quenching and work-up with 10 % KH_2PO_4 solution and adjustment of the pH to pH=6. Crude product was extracted the aqueous with DCM, and then solvent was evaporated and replaced with EtOH and this process was repeated to provide a crude slurry in EtOH. Crystallization from EtOH as solvent provided 3-(1-oxo-5-(((1S,2S)-2-(3-phenylazetidin-1-yl)cyclohexyl)oxy)isoindolin-2-yl)piperidine-2,6-dione in 99.1% purity in 58% isolated yield.

CLAIMS:

1. A crystalline form of Compound I:



I,

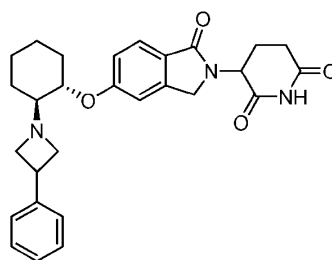
(Compound I Form A), wherein Compound I Form A is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 13.7 , 17.5 , and $21.4^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

2. The crystalline form of claim 1, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 10.9 , 15.5 , or $16.4^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 1;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 207.1°C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 2;
- v) thermogravimetric analysis (TGA) showing a weight loss of about $0.6\text{ wt\%}$ up to 150°C ; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in

FIG. 3.

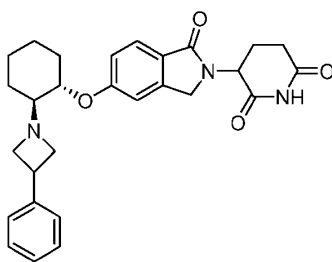
3. An amorphous form of Compound I:



I,

(Compound I Amorphous Form), wherein Compound I amorphous Form is characterized by an X-ray powder diffractogram substantially as shown in FIG. 4.

4. A fumarate salt of Compound I:



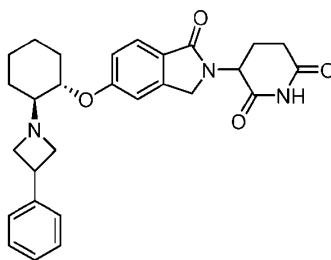
I.

5. The crystalline form of fumarate salt of Compound I according to claim 4 (Compound I fumarate salt Form A), wherein Compound I fumarate salt Form A is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 10.0, 18.1, and 23.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

6. The crystalline form of claim 5, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 5.6, 19.7, or 21.8 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 5;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 16.1 $^\circ\text{C}$ (onset temperature) and an endotherm at about 120.0 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 6;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.5 wt% up to 120 $^\circ\text{C}$, and a further weight loss of about 8.3 wt% from 120 $^\circ\text{C}$ up to 173 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 7.

7. A L-tartrate salt of Compound I:

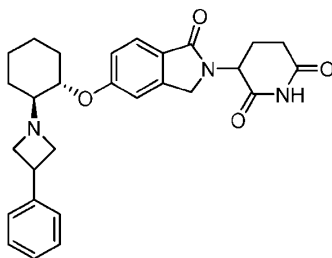


I.

8. The crystalline form of L-tartrate salt of Compound I according to claim 7 (Compound I L-tartrate salt Form A), wherein Compound I L-tartrate salt Form A is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.9, 17.0, and 22.3 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

9. The crystalline form of claim 8, further characterized by:
- i) one or more peaks ($\pm 0.2^\circ$) at 12.0, 18.2, or 20.0 $^\circ 2\theta$;
 - ii) a diffractogram substantially as shown in FIG. 8;
 - iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 16.1 $^\circ\text{C}$ (onset temperature) and an endotherm at about 138.2 $^\circ\text{C}$ (onset temperature);
 - iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 9;
 - v) thermogravimetric analysis (TGA) showing a weight loss of about 5.1 wt% up to 90 $^\circ\text{C}$; or
 - vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 10.

10. A nicotinamide co-crystal of Compound I:

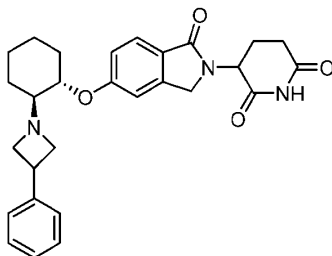


I.

11. The crystalline form of nicotinamide co-crystal of Compound I according to claim 10, (Compound I nicotinamide co-crystal), wherein Compound I nicotinamide co-crystal is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 14.8, 25.9, and 27.3 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

12. The crystalline form of claim 11, further characterized by:
- i) one or more peaks ($\pm 0.2^\circ$) at 7.3, 18.3, or 19.5 $^\circ 2\theta$; or
 - ii) a diffractogram substantially as shown in FIG. 11.

13. A succinate salt of Compound I:



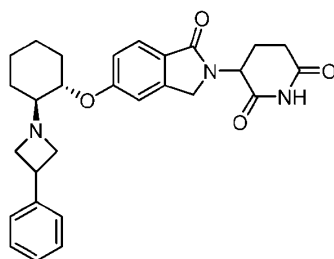
I.

14. The crystalline form of succinate salt of Compound I according to claim 13 (Compound I succinate salt Form A), wherein Compound I succinate salt Form A is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 3.7, 11.2, and 16.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

15. The crystalline form of claim 14, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 13.7, 17.7, or 21.6 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 12;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 14.3 $^\circ\text{C}$ (onset temperature) and an endotherm at about 90.9 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 13;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.4 wt% up to 82 $^\circ\text{C}$, and a further weight loss of about 4.0 wt% from 82 $^\circ\text{C}$ up to 160 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 14.

16. A *R*-mandelate salt of Compound I:



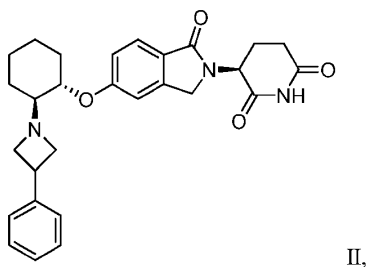
17. The crystalline form of *R*-mandelate salt of Compound I according to claim 16 (Compound I *R*-mandelate salt Form A), wherein Compound I succinate salt Form A is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 8.8, 14.6, and 19.8 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

18. The crystalline form of claim 17, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 4.4, 7.3, or 21.2 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 15;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 101.3 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 16;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 5.0 wt% up to 141 $^\circ\text{C}$; or

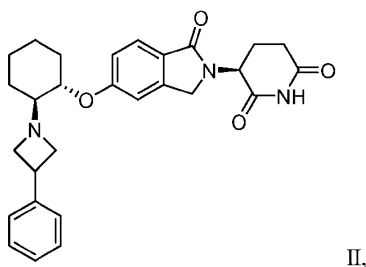
vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 17.

19. An amorphous form of Compound II:



(Compound II Amorphous Form), wherein Compound II amorphous Form is characterized by an X-ray powder diffractogram substantially as shown in FIG 18.

20. A crystalline form of Compound II:



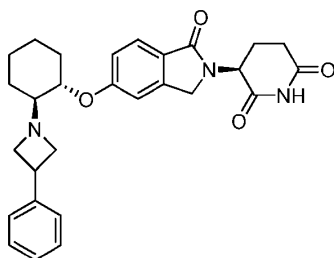
(Compound II Form B), wherein Compound II Form B is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.3, 17.8, and 19.0 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

21. The crystalline form of claim 19, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 12.3, 21.5, or 22.0 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 19;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 104.4 $^\circ\text{C}$ (onset temperature), and endotherm at about 135.3 $^\circ\text{C}$ (onset temperature), and an endotherm at about 197.0 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 20;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 19.9 wt% up to 138 $^\circ\text{C}$, and a further weight loss of about 3.7 wt% from 138 $^\circ\text{C}$ up to 196 $^\circ\text{C}$; or

vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 21.

22. A crystalline form of Compound II:



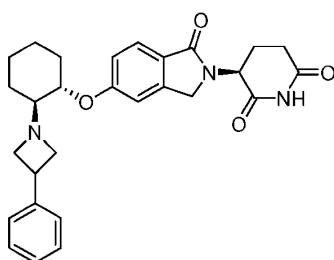
II,

(Compound II Form C), wherein Compound II Form C is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 4.0, 12.0, and 14.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

23. The crystalline form of claim 22, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 9.6, 16.5, or 19.3 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 22;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 205.6 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 23;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 2.1 wt% up to 180 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 24.

24. A crystalline form of Compound II:



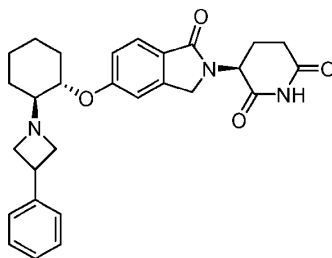
II,

(Compound II Form D1), wherein Compound II Form D1 is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4, 7.9, and 15.9 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

25. The crystalline form of claim 24, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 12.3, 17.9, or $20.6^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 25;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 74.1°C (onset temperature), and an endotherm at about 205.3°C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 26;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 2.7 wt% up to 85°C , and a further weight loss of about 5.5 wt% from 85°C up to 134°C ; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 27.

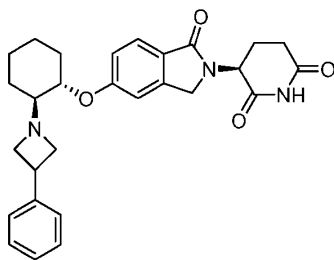
26. A crystalline form of Compound II:



II,

(Compound II Form D2), wherein Compound II Form D2 is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4° , 16.2° , and $17.9^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

27. The crystalline form of claim 26, further characterized by:
- i) one or more peaks ($\pm 0.2^\circ$) at 4.0° , 8.1° , and $19.2^\circ 2\theta$;
 - ii) a diffractogram substantially as shown in FIG. 28;
 - iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 31.7°C (onset temperature), an endotherm at about 79.2°C (onset temperature), an endotherm at about 113.8°C (onset temperature), an exotherm at about 156.4°C (onset temperature), and an endotherm at about 194.6°C (onset temperature); or
 - iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 29.
28. A crystalline form of Compound II:

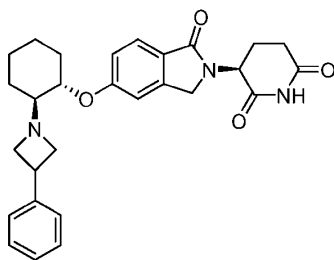


II,

(Compound II Form D3), wherein Compound II Form D3 is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.4 , 8.1 , and $16.2^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

29. The crystalline form of claim 28, further characterized by:
- i) one or more peaks ($\pm 0.2^\circ$) at 12.8 , 8.0 , or $20.7^\circ 2\theta$;
 - ii) a diffractogram substantially as shown in FIG. 30;
 - iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 65.9°C (onset temperature) and an endotherm at about 124.8°C (onset temperature);
 - iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 31;
 - v) thermogravimetric analysis (TGA) showing a weight loss of about $4.5\text{ wt}\%$ up to 117°C , and a further weight loss of about $0.7\text{ wt}\%$ from 117°C up to 250°C ; or
 - vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 32.

30. A crystalline form of Compound II:



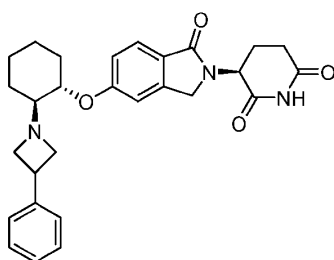
II,

(Compound II Form E), wherein Compound II Form E is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 6.1 , 7.6 , and $15.2^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

31. The crystalline form of claim 30, further characterized by:
- i) one or more peaks ($\pm 0.2^\circ$) at 12.2 , 13.5 , or $21.2^\circ 2\theta$;
 - ii) a diffractogram substantially as shown in FIG. 33;

- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 12.6 °C (onset temperature), an endotherm at about 93.4 °C (onset temperature), and an endotherm at about 197.0 °C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 34;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 2.0 wt% up to 80 °C, and a further weight loss of about 7.3 wt% from 80 °C up to 140 °C; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 35.

32. A crystalline form of Compound II:

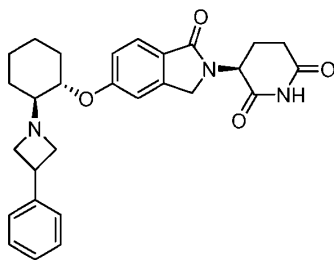


II,

(Compound II Form F), wherein Compound II Form F is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.2, 18.0, and 19.2 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

33. The crystalline form of claim 32, further characterized by:
- i) one or more peaks ($\pm 0.2^\circ$) at 14.1, 17.4, or 20.1 $^\circ 2\theta$;
 - ii) a diffractogram substantially as shown in FIG. 36;
 - iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 99.4 °C (onset temperature) and an endotherm at about 208.7 °C (onset temperature);
 - iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 37;
 - v) thermogravimetric analysis (TGA) showing a weight loss of about 0.8 wt% up to 95 °C, and a further weight loss of about 14.2 wt% from 95 °C up to 140 °C; or
 - vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 38.

34. A crystalline form of Compound II:



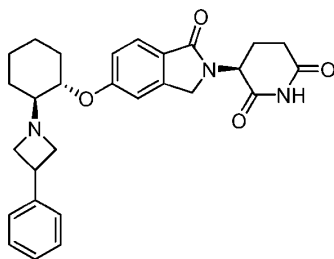
II,

(Compound II Form G), wherein Compound II Form G is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.3, 18.4, and 19.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

35. The crystalline form of claim 34, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 11.1, 14.3, or 16.9 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 39;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 45.4 $^\circ\text{C}$ (onset temperature), an endotherm at about 105.6 $^\circ\text{C}$ (onset temperature), and an exotherm at about 177.0 $^\circ\text{C}$ (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 40;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 6.0 wt% up to 150 $^\circ\text{C}$, and a further weight loss of about 4.3 wt% from 150 $^\circ\text{C}$ up to 250 $^\circ\text{C}$; or
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 41.

36. A crystalline form of Compound II:



II,

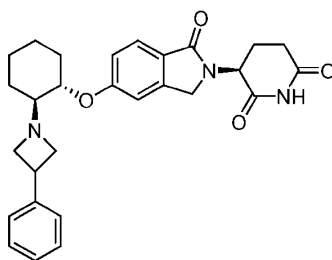
(Compound II Form H), wherein Compound II Form H is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 7.2, 18.1, and 21.6 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

37. The crystalline form of claim 36, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 11.0, 14.9, or 18.2 $^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 42;

- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 117.9 °C (onset temperature) and an exotherm at about 179.2 °C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 43;
- v) thermogravimetric analysis (TGA) showing a weight loss of about 3.6 wt% up to 115 °C, and a further weight loss of about 8.1 wt% from 115 °C up to 150 °C;
- vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 44;
- vii) unit cell parameters: $a = 24.1274$ (6), Å, $b = 24.1274$ (6) Å, $c = 13.0308$ (4) Å, $V = 6569.4$ (4) Å³;
- viii) unit cell parameters: $a = 24.2881$ (6), Å, $b = 24.2881$ (6) Å, $c = 13.0707$ (4) Å, $V = 6677.5$ (4) Å³; or
- ix) unit cell parameters: $a = 24.2722$ (6), Å, $b = 24.2711$ (6) Å, $c = 13.0585$ (4) Å, $V = 6662.6$ (4) Å³.

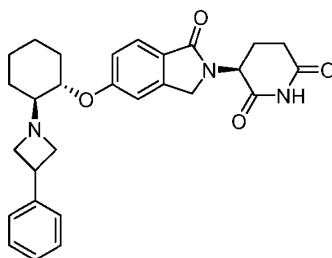
38. A crystalline form of Compound II:



(Compound II Form I), wherein Compound II Form I is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 15.4, 15.8, and 21.9 °2 θ as determined on a diffractometer using Cu-K α radiation.

39. The crystalline form of claim 38, further characterized by:
- i) one or more peaks ($\pm 0.2^\circ$) at 7.0, 20.3, or 21.2 °2 θ ;
 - ii) a diffractogram substantially as shown in FIG. 48;
 - iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 94.1 °C (onset temperature) and an endotherm at about 186.2 °C (onset temperature);
 - iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 49;
 - v) thermogravimetric analysis (TGA) showing a weight loss of about 15.9 wt% up to 91 °C, a further weight loss of about 18.1 wt% from 91 °C up to 179 °C, and a further weight loss of about 7.8 wt% from 179 °C up to 261 °C; or
 - vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 50.

40. A crystalline form of Compound II:



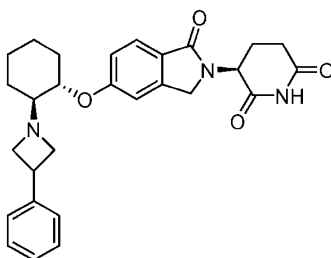
II,

(Compound II Form J), wherein Compound II Form J is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 17.2 , 17.6 , and $20.4^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

41. The crystalline form of claim 40, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 7.2 , 18.3 , or $19.0^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 51;
- iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 82.3°C (onset temperature), an endotherm at about 103.0°C (onset temperature), an endotherm at about 127.1°C (onset temperature), and an endotherm at about 214.9°C (onset temperature);
- iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 52;

42. A fumarate salt of Compound II:



II.

43. The crystalline form of fumarate salt of Compound II according to claim 42 (Compound II fumarate salt Form B), wherein Compound II fumarate salt Form B is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 9.9 , 19.3 , and $20.0^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

44. The crystalline form of claim 43, further characterized by:

- i) one or more peaks ($\pm 0.2^\circ$) at 16.5 , 18.6 , or $23.0^\circ 2\theta$;
- ii) a diffractogram substantially as shown in FIG. 53;

iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 19.8 °C (onset temperature), an endotherm at about 118.2 °C (onset temperature), an endotherm at about 181.7 °C (onset temperature), an exotherm at about 195.5 °C (onset temperature), and an endotherm at about 200.7 °C (onset temperature);

iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 54;

v) thermogravimetric analysis (TGA) showing a weight loss of about 3.4 wt% up to 165 °C, and a further weight loss of about 7.8 wt% from 165 °C up to 250 °C; or

vi) thermogravimetric analysis (TGA) comprising a thermogram substantially as shown in FIG. 55.

45. The crystalline form of fumarate salt of Compound II according to claim 42 (Compound II fumarate salt Form C), wherein Compound II fumarate salt Form C is characterized by an X-ray powder diffractogram comprising peaks ($\pm 0.2^\circ$) at 9.6, 17.5, and 18.1 $^\circ 2\theta$ as determined on a diffractometer using Cu-K α radiation.

46. The crystalline form of claim 45, further characterized by:

i) one or more peaks ($\pm 0.2^\circ$) at 14.9, 19.6, or 21.1 $^\circ 2\theta$;

ii) a diffractogram substantially as shown in FIG. 56;

iii) a differential scanning calorimetry (DSC) curve comprising an endotherm at about 19.7 °C (onset temperature), an endotherm at about 99.2 °C (onset temperature), and an endotherm at about 161.5 °C (onset temperature); or

iv) a differential scanning calorimetry (DSC) curve substantially as shown in FIG. 57.

47. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a compound of any of the preceding claims, and a pharmaceutically acceptable excipient.

48. A method for modulating cereblon activity, which method comprises contacting cereblon with an effective amount of a compound of any one of claims 1-46, or a pharmaceutically acceptable salt, solvate, stereoisomer, or tautomer thereof, under conditions wherein cereblon is modulated.

49. A method for degrading IKZF2, which method comprises contacting IKZF2 with an effective amount of a compound of any one of claims 1-46, or a pharmaceutically acceptable salt, solvate, stereoisomer, and/or tautomer thereof, under conditions wherein IKZF2 is degraded.

50. A method to degrade IKZF2 in a subject, which method comprises administering to said subject an effective amount of a compound according to any one of claims 1-46, or a pharmaceutically acceptable salt, solvate, stereoisomer, and/or tautomer thereof.

51. A method to treat cancer in a subject in need thereof, which method comprises selecting a subject whose cancer is mediated at least in part by IKZF2 and administering to said subject an effective amount of a compound of any one of claims 1-46, or a pharmaceutically acceptable salt, solvate, stereoisomer, and/or tautomer thereof.

52. A method to treat cancer in a subject in need thereof, which method comprises selecting a subject whose cancer is mediated at least in part by IKZF2 and administering to said subject an effective amount of a pharmaceutical composition of claim 47.

53. The method of claim 51 or 52, wherein the cancer is non-small cell lung cancer (NSCLC), melanoma, triple-negative breast cancer (TNBC), nasopharyngeal cancer (NPC), microsatellite stable colorectal cancer (mssCRC), thymoma, carcinoid, acute myelogenous leukemia, or gastrointestinal stromal tumor (GIST).